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RELATIVE BIO-EQUIVALENCE OF SALBUTAMOL MDIs WITHOUT AND WITH THE ATTACHED SPACERS

Development and validation of novel HPLC methods for the determination of salbutamol (and terbutaline) in urine excreted post-inhalation for bioequivalence and pharmacokinetic studies of Salbutamol MDIs

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Relative Bio-Equivalence of Salbutamol MDIs Without and With the Attached Spacers
Key words: Salbutamol HFA MDI, Bioequivalence, Urinary Pharmacokinetics, HPLC, SPE, Spacer, Charcoal Block, *In-Vitro* Equivalence, FPD, TDD

Abstract

This research explored *in-vitro* and *in-vivo* performance of three salbutamol metered dose inhalers (MDIs): Ventolin Evohaler (Evo), Airomir (Airo) and Salamol. In the *in-vitro* studies, critical quality attributes of the MDI using an Andersen cascade impactor (ACI) were examined and included measurement of fine particle dose (FPD) and total delivered dose (TDD). Bioequivalence studies were conducted in humans using the urinary pharmacokinetic method. Post-inhalation urinary excretion of salbutamol in the first 0.5 hour (lung deposition, USAL0.5) and over 24 hours (total systemic bioavailability, USAL24) were compared to determine the bioequivalence of the MDIs. The spacers recommended for use with these inhalers were also studied, and charcoal block studies were performed to assess the extent of USAL0.5.

The three MDIs had FPD (µg) of 78, 91 and 89, respectively; the latter pair was equivalent. Their USAL0.5 (6, 7 & 7 µg) was however not bioequivalent. These MDIs delivered equivalent dose (177, 174 & 180 µg) which reflected on their USAL24 (101, 84 & 97 µg). Nevertheless, USAL24 was inequivalent between Evo and Airo.

The FPD of Evo with Volumatic (VOL), AeroChamber Plus (AERO) and Able spacer was 78, 68 and 74 µg, respectively. The AERO treatment method was not equivalent to the MDI while VOL and Able were equivalent between them. Spacer USAL0.5 (16, 15 & 14 µg) was not bioequivalent to the MDI but to each other. The spacer *in-vitro* TDD (95, 85 & 92 µg) was inequivalent to the MDI treatment method. In contrast, their USAL24 was bioequivalent (97, 85 & 90 µg).

The FPD of Airomir with AERO (95 µg) was *in-vitro* equivalent while USAL0.5 (15 µg) of this treatment method was bio-inequivalent to the MDI alone. On the contrary, the TDD (110 µg) and USAL24 (84 µg) of AERO were respectively *in-vitro* inequivalent and bioequivalent to the MDI alone.

The FPD (µg) of Salamol MDI alone and with VOL (84) and AERO (86) as well as between the spacers was equivalent. However, the USAL0.5 of the MDI was not bioequivalent to spacers (20 and 18 µg) despite being equivalent between the spacers. In contrast, the respective TDD (103 and 95 µg) of spacer treatment methods were *in-vitro* inequivalent to the MDI alone albeit having bioequivalent USAL24 (86 and 87 µg).

The variations in the *in-vitro* performance of the three MDIs are most likely due to differences in their formulations and designs. As the performance metrics of the MDI influence lung deposition, substituting one MDI with another can have clinical implications.

Although the spacers reduced *in-vitro* TDD of the MDI to about half, their use increased lung deposition by over two folds, the magnitude of which varied with the MDI and spacer type. Despite significant decrease in dose delivery, the total systemic bioavailability with the spacers was similar to that with the MDI alone. This systemic bioequivalence is more likely due to greater USAL0.5 with the spacers. The results of the charcoal block studies reinforced this outcome.

The present study is unique as it used a clinically relevant salbutamol MDI dose (two puffs), assessed results for equivalence and analysed ACI deposition data further as stage groups. The deposition on adjacent ACI stages were grouped together as coarse, fine and extra-fine particle masses to identify their more likely deposition sites in the human respiratory tract. Moreover, this thesis describes highly sensitive and novel HPLC and SPE methods, developed and validated to quantify salbutamol in urinary and aqueous matrices.

As the clinical effects of MDIs are related to their lung deposition, the current work emphasizes the importance of spacer use. Nevertheless, differences in dose delivery between spacers may have clinical consequences. Hence, only the specific spacer recommended for use with the MDI should be used.

Publications

Sections of this thesis have already been published in the following form:

1. Mazhar, S. H. R. A. and Chrystyn, H. (2009) New HPLC assay for urinary salbutamol concentrations in samples collected post-inhalation. *Journal of Pharmaceutical and Biomedical Analysis*, 50(2), 175 – 182. https://doi.org/10.1016/j.jpba.2009.04.006.

2. Mazhar, S. H. R. A. and Chrystyn, H. (2008) Salbutamol relative lung and systemic bioavailability of large and small spacers. *Journal Pharmacy and Pharmacology*, 60(12), 1609 – 1613. https://doi.org/10.1211/jpp.60.12.0006.

3. Mazhar, S. H. R., Ismail, N. E., Newton, D. A. G. and Chrystyn, H. (2008) Relative lung deposition of salbutamol following inhalation from a spacer and a Sidestream jet nebuliser following an acute exacerbation. *British Journal of Clinical Pharmacology*, 65(3), 334 – 337. https://doi.org/10.1111/j.1365-2125.2007.03036.x.
4. *In-vitro* and *in-vivo* correlation of salbutamol MDIs without and with spacers. In preparation.

Dedication

To Prophet Mohammed (peace be upon him and his progeny), the city of knowledge, and Imam Ali Ibn Abi Talib (peace be upon him), the gate of the knowledge.

To my mother Syeda Nadira Khatoon and father Syed Iqtidar Ali Mazhar who longed-for me achieving this educational objective but could not live long enough to see this happening! May they rest in peace!

To my deceased Uncle Syed Mohammad Naqvi and Aunty Farzana Munawar Naqvi, who looked after me like my parents. May they rest in peace!

To my brothers and sisters who have always been a source of encouragement, motivation and inspiration.

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List of Abbreviations

Acronym	Explanation
ABLE	Able Spacer
ACI	Anderson Cascade Impactor
Actu	Actuator
AERO	AeroChamber Plus
Airo	Airomir
ANOVA	Analysis of Variance
APSD	Aerodynamic Particle Size Distribution
AUC	Area Under Curve
BE	Bioequivalence
BNF	British National Formulary
BP	British Pharmacopoeia
BTS	British Thoracic Society
C or +c	Charcoal
Can	Canister
C_{av}	Average Concentration
CFC	Chlorofluorocarbon
CI	Confidence intervals
CITDAS	Copley Inhaler Testing Data Analysis Software
C_{max}	Maximum Concentration
COPD	Chronic Obstructive Pulmonary Disease
CPM	Coarse Particle Mass. Deposition on ACI stages S0, S1 and S2
CQA	Critical Quality Attribute
DPI	Dry Powder Inhaler
DUSA	Dosage Unit Sampling Apparatus
EDU	Emitted Dose Uniformity
EPM	Extrafine Particle Mass. Deposition on ACI stages S6, S7 and Filter
EU	European Union
Evo	Ventolin Evohaler
Evohaler	Ventolin Evohaler
FDA	Food and Drug Administration
FEV_1	Forced Expiratory Volume in one second
FPD	Fine Particle Dose
FPF	Fine Particle Fraction

Acronym	Explanation
FPF%	Fine Particle Fraction as a percentage
FPM	Fine Particle Mass. Deposition on ACI stages S3, S4 and S5
GI	Gastrointestinal
GINA	Global Initiative for Asthma
GIT	Gastrointestinal Tract
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GSD	Geometric Standard Deviation
GSK	GlaxoSmithKline
h	hour
HFA	Hydrofluoroalkane
HLB	Hydrophilic Lipophilic Balance
HPLC	High Performance Liquid Chromatography
HRT	Human Respiratory Tract
ICH	International Committee of Harmonisation
ICS	Inhaled Corticosteroid
IP	Induction Port
IP+CPM	Deposition on IP and ACI stages S0, S1 & S2; represents non-respirable dose.
IPM	Induction Port Mass
IS	Internal standard
LABA	Long Acting Beta-Agonist
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
LOQ	Limit of Quantification
MDI	Metered Dose Inhaler
MDI+SP	MDI attached to a spacer
MHRA	Medicines and Healthcare Products Regulatory agency
Min	Minute
MMAD	Mass Median Aerodynamic Diameter
MP	Mobile Phase
MSLI	Multistage Liquid Impinger
NC or nc	No Charcoal
ND	Nominal Dose
NGI	New Generation Impactor
NHS	National Health Service

Acronym	Explanation
NICE	National Institute for Health and Clinical Excellence
PD	Pharmacodynamics
PEF	Peak Expiratory Flow
PEFR	Peak Expiratory Flow Rate
PIL	Patient Information Leaflet
Ph. Eur.	European Pharmacopoeia
PK	Pharmacokinetic
QC	Quality Control
RSD	Relative Standard Deviation
S	Stage of ACI
S0toF	Impactor mass; deposition on ACI stages S0 to S7 & Filter
Sala	Salamol
SAS	Salbutamol Aqueous Standard
SD	Standard Deviation
Sec	Second
SIGN	Scottish Intercollegiate Guidelines
SMI	Soft Mist Inhaler
SP+IP+CPM	Deposition on spacer, IP and ACI stages S0, S1 & S2; represents non-respirable dose.
Spacer	Chambered add-on device for use with an MDI
SPC	Summary of Product Characteristics
SPE	Solid Phase Extraction
SUS	Salbutamol urine standard
TDD	Total Delivered Dose
TED	Total Emitted dose
t_{max}	Time to reach the maximum concentration
TMI	Trudell Medical International
TRD	Total Recovered Dose
USAL METHOD	SPE Method for free (unchanged) salbutamol
USAL	Un-hydrolysed Salbutamol Urinary Standard
USAL0.5	Salbutamol excretion in urine in the first 0.5 hour
USAL0.5C	Salbutamol excretion in urine in the first 0.5 hour with charcoal intake
USAL24	Total salbutamol excretion in urine during 24 hours (includes both active and metabolised moieties)
USAL24C	Total salbutamol excretion in urine during 24 hours with charcoal

Acronym	Explanation
	intake (includes both active and metabolised moieties)
USAL24Post	Active and metabolised salbutamol excretion in urine during 24 hours
USAL24PostC	Active and metabolised salbutamol excretion in urine during 24 hours with charcoal intake
USAL24PostNC	Active and metabolised salbutamol excretion in urine during 24 hours without charcoal intake
USAL24Pre	Active salbutamol excretion in urine during 24 hours
USAL24PreC	Active salbutamol excretion in urine during 24 hours with charcoal intake
USAL24PreNC	Active salbutamol excretion in urine during 24 hours without charcoal intake
USALMET METHOD	SPE Method for total salbutamol (free and metabolised)
USALMET	Hydrolysed Salbutamol Urinary Standard
USALMET	Salbutamol Metabolites in urine
USALMETc	Metabolised salbutamol excretion in urine during 24 hours with charcoal intake
USALMETnc	Metabolised salbutamol excretion in urine during 24 hours without charcoal intake
USP	United State Pharmacopoeia
UV	Ultraviolet
v/v	Volume by Volume
VHC	Valved Holding Chamber
VOL	Volumatic
Vs	Versus
WMA	World Medical Association

1 Chapter 1: Introduction

1.1 Overview

Inhaled drug delivery is the mainstay of managing respiratory ailments such as asthma and COPD. The inhalation route is a fast and effective way of delivering drugs to the site of action for a localised effect which leads to a rapid clinical response, particularly for inhaled β-agonist therapy (Dolovich and Dhand, 2011; Cheng, 2014; Lavorini et al., 2014; Bonini and Usmani, 2015; Lewis, 2015; Ivey et al., 2015). This route of drug administration enables delivery of low doses directly to the airways which minimises systemic side-effects while also avoiding the first-pass metabolism with minimum reduction of bioavailability. The large surface area and highly permeable air-to-blood barrier of the respiratory system make it a highly receptive site for drug delivery (Demoly et al., 2014).

Respiratory drug delivery can be achieved by a number of devices such as Metered Dose Inhalers (MDIs), Dry Powder Inhalers (DPIs), Soft Mist Inhalers (SMIs) and nebulisers. These methods of delivery are diversifying with technological development and changing patients' needs. The MDI has been in use for 60 years and presently represents the most common method of drug delivery to the lungs (Ivey et al., 2015).

The clinical effects of an asthma treatment have been shown to be directly correlated with the drug's lung deposition (Newman, 2000). Pulmonary drug deposition of an inhaled drug is influenced by the aerodynamic particle size distribution of the emitted aerosol (Seale and Harrison, 1998; Darquenne, 2012). Therefore, *in-vitro* measurements of critical performance parameters of an MDI will reflect on the likely success of drug delivery and targeted deposition (Lewis, 2015). These include assessing the total quantity of drug emitted from the MDI and therefore available to the patient, and the respirable dose and aerodynamic size of the particles that make up the emitted aerosol. These performance metrics impact the proportion of the total dose that reaches the lungs during inhalation, along with its regional intrapulmonary deposition, and hence clinical effects.

These *in-vitro* MDI performance metrics are measured using cascade impactors such as ACI (Mitchell et al., 2007). These equipments simulate particle deposition in the human airways (BP, 2005; USP28-NF23, 2005; Ph. Eur, 2011; Cheng, 2014) (Figure 2.3.1). They provide information on how formulation and device variables affect MDI performance besides estimating its dose delivery efficiency. Hence, ACI has been used

in this research project to evaluate and compare *in-vitro* performance of salbutamol HFA MDIs.

Spacer used with an MDI assists patients in circumventing the problem of coordination in press and breathe manoeuvres of the discharged dose. Hence, the effects of this treatment method on the performance metrics of salbutamol MDIs will also be explored.

Lung deposition and total systemic bioavailability of salbutamol MDIs can be effectively determined and compared with urinary pharmacokinetic (PK) studies in healthy volunteers (EMA, 2009 & 2010). The method developed by Hindle and Chrystyn (1992) will be used for this purpose (see Chapter 3 Methodology). Each study is complemented by charcoal blockade element to identify the proportion of the dose delivered to the lungs.

Regulatory authorities require that *in-vitro* studies are to be carried out to assess any claims of equivalence between MDIs. These authorities recommend using cascade impactors to identify similarity in their performance metrics. If these MDIs are not found *in-vitro* equivalent, EMA (2006, 2009) allows proving equivalence in PK studies. Nevertheless, both *in-vitro* and *in-vivo* equivalence studies will be carried out in this project to explore if their outcomes are co-related.

1.2 Aims and objectives

1.2.1 Aims

- To evaluate *in-vitro* and *in-vivo* equivalence of salbutamol HFA MDIs without and with spacer.

1.2.2 Objectives

I. To develop and validate HPLC methods for the determination of salbutamol in aqueous samples collected from *in-vitro* studies with ACI and in human urine samples collected following inhalation.
II. To undertake *in-vitro* characterisation of salbutamol HFA MDIs using ACI.
III. To investigate the relative lung and total systemic bioavailability of salbutamol HFA MDIs in healthy volunteers using urinary pharmacokinetics.

1.2.3 Framework

The aims and objectives of the research will be achieved in three parts (Figure 1.3.1):

I. Analytical Method Development and Validation
   a. To develop and validate efficient, robust and reliable solid phase extraction (SPE) methods for the separation and concentration of unhydrolysed and hydrolysed salbutamol from human urine samples collected post-inhalation.
   b. To develop and validate a sensitive and robust HPLC method for quantifying salbutamol in aqueous samples collected from *in-vitro* studies with ACI and residual dose in the MDI components and spacers following administrations to human subjects.

II. Determination of *In-Vitro* Equivalence
   a. This study will have two components-
      (i) *In-vitro* characterisation of salbutamol HFA MDIs used alone. MDIs included in this study are Ventolin Evohaler®, Airomir® and Salamol®.
      (ii) *In-vitro* characterisation of salbutamol HFA MDIs used with spacer. The spacers selected for this project are Volumatic, AeroChamber Plus and Able spacer.

III. Determination of Bioequivalence

   These studies will have two components, each of which will have two parts-
   a. Determination of bioavailability of salbutamol MDIs used alone.
   b. Determination of bioavailability of salbutamol MDIs used with spacers.
      (ii)

1.3 Thesis Structure

This introductory chapter is followed by review of previous work and, current and ongoing issues related to this research project in Chapter 2. The rationale for methodological approach is highlighted and discussed in Chapter 3.

Development and validation of HPLC and SPE methods have been described in Chapter 4.

The *in-vitro* and *in-vivo* equivalence studies have been put together for each treatment method evaluated in this project. Hence, these equivalence studies for salbutamol MDI alone, Ventolin Evohaler without and with spacers, Airomir without and with spacer and Salamol without and with spacers have been separately explored in Chapters 5, 6, 7 and 8, respectively.

General discussion and overall conclusions drawn from this project and direction for future work are included in Chapter 9.

The organogram of the thesis is shown in Figure 1.3.1.

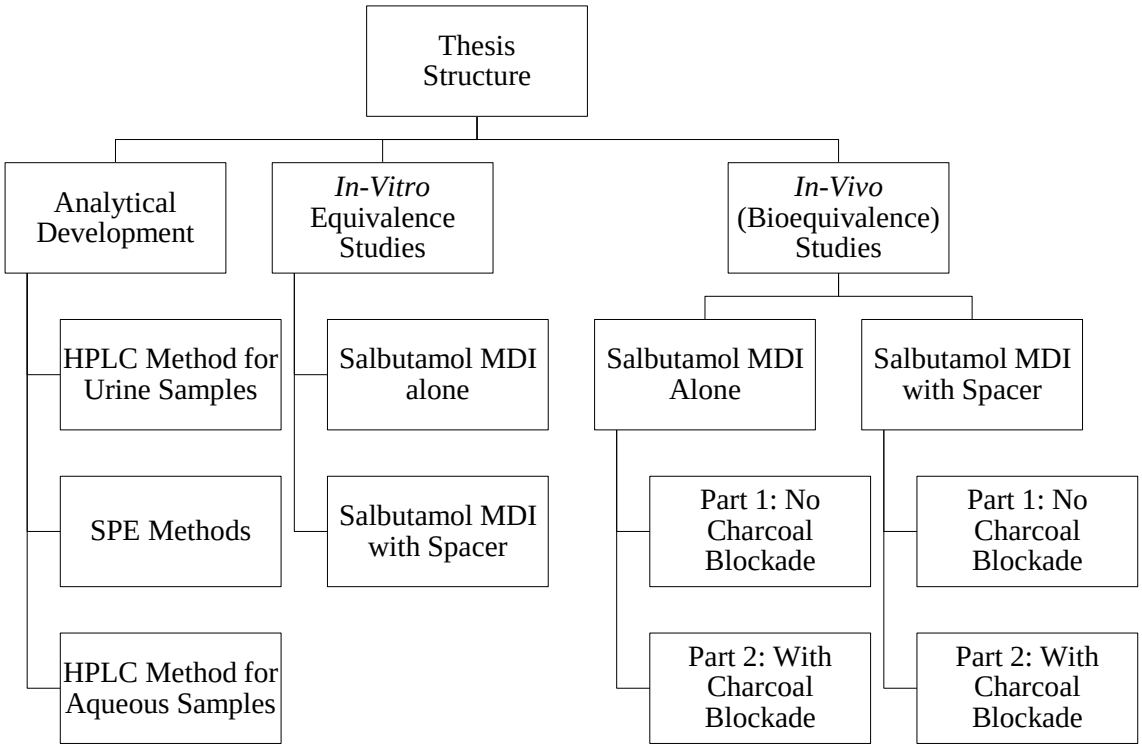


Figure 1.3.1. Thesis structure.

2 Chapter 2: Literature Review

2.1 Overview

Asthma and COPD are amongst the most prevalent respiratory diseases (GINA, 2017; GOLD, 2018). Inhalation therapy is the mainstay of providing relief from these ailments (NICE, 2010 & 2017). The pulmonary route allows drug delivery directly to their target sites which can result in a rapid onset of their activity. This is highly desirable, for instance when delivering bronchodilators for the treatment of asthma. Additionally, this localised delivery minimises systemic exposure and, thus, potential side-effects (Roche et al., 2013). This targeted drug delivery is achieved by a number of inhalation devices which include inhalers and nebulisers. Selective β_2 agonists and corticosteroids are the cornerstone of treatment for these diseases. MDI remains the delivery device of choice (Lavorini et al., 2011), and salbutamol is the most widely prescribed β_2 agonist (GINA, 2017).

MDIs originally contained chloroflourocarbons (CFCs) as propellants. However, due to their deleterious effects on the ozone layer (Molina and Rowland, 1974), these were gradually replaced with hydrofluoroalkane (HFA) propellants in compliance to the Montreal Protocol (UNEP, 2017). Pharma industry faced great challenges during this phase-out period (Smith, 1995; Atkins, 1999; Bowman and Greenleaf, 1999; Cummings, 1999) which incurred significant investment in research and development (Leach, 2005; Stein and Thiel, 2017). Nevertheless, this was in disguise an opportunity to develop and modify MDI device components and reformulate drugs in HFA propellants.

Many patients have difficulty in coordinating MDI actuation with inhalation which can be overcome with a spacer. Nonetheless, the combination of MDI with a spacer produces a different and new dose delivery system, the efficiency of which is likely to be determined by the characteristics of its component two devices. It has been shown that the delivery of MDI dose is governed by device design and formulation (Ross and Gabrio, 1999; Gabrio et al., 1999, Cripps et al., 2000; Stein et al., 2014; Myrdal et al., 2014) and spacer characteristics, such as geometry, volume and construction material, can significantly influence this (Barry and O'Callaghan, 1995a, 1996 & 1997; Lipworth and Clark, 1998a; Mitchell et al., 1999; Rau et al., 2006; Oliveira et al., 2015 & 2016). Therefore, the technologically modified and reformulated salbutamol HFA MDIs are likely to have intrinsically varied drug delivery to the lungs when used alone or with a spacer. Most of the previous *in-vitro* and *in-vivo* studies compared salbutamol HFA

MDIs to those of CFC MDIs during their transition period. Later *in-vitro* studies were conducted to assess the suitability of marketed spacers for salbutamol HFA MDIs (Mitchell et al., 1999; Hatley et al., 2014; Slator et al., 2014; Johnson et al., 2016) or to explore their plume characteristics (Brambilla et al., 2011; McCabe et al., 2012; Hautmann et al., 2013; Kunda et al., 2017). The present work focuses on three differently formulated and designed salbutamol HFA MDIs, vis-à-vis: Ventolin Evohaler®, Airomir® and Salamol® (Table 2.2.1). To the knowledge of this author, these HFA MDIs have not been compared *in-vitro* with each other independently, in particular with spacers, and that studies on these MDIs have not been complemented by *in-vivo* studies. Nevertheless, *in-vitro* studies for HFA MDIs alone and with a cardboard spacer have recently been reported by Johnson et al. (2016).

In this chapter, a brief backgrounder is followed up with a review of *in-vitro* studies on salbutamol HFA MDIs. While these studies encompass broad areas, primary focus remains on salbutamol HFA MDI when used alone and with a spacer. Further, review of *in-vitro* studies centres on particle size characterisation using Andersen Cascade Impactor (ACI) and Next Generation Impactor (NGI) due to their similarity with each other (Mitchell et al., 2003; Kamiya et al., 2004) and because ACI will be used in this project. *In-vivo* pharmacokinetic studies have been reviewed in Chapter 3 (Methodology). An overview of literature on HPLC and SPE method development for salbutamol quantitation in urine post-inhalation has been provided in Chapter 4.

2.2 The Rejuvenated MDI

The MDI has now been available for over 60 years. The technology has evolved significantly over these years particularly since transition from CFC to HFA containing formulations. However, this transition was not straightforward as HFAs could not directly replace CFC propellants due to incompatibility of HFA formulations with previously used excipients and MDI device components (Myrdal et al., 2014). As a result, significant efforts were put to develop new device components which were complemented with varying formulation approaches. Notable modifications to the MDI device components (

Figure 2.2.1) included development of new elastomers, redesigned valves, changes in nozzle diameter and metered dose volume, coated canisters and actuator (Leach, 2005; Bell and Newman, 2007; Roche and Dekhuijzen, 2016; Stein and Thiel, 2017).

Two general approaches were applied for CFC to HFA transition vis-à-vis: to reproduce the similar dose delivery characteristics of the original MDI or to overhaul the existing ones (Table 2.2.1). The first approach is mirrored in the preservation of Ventolin CFC characteristics into Ventolin Evohaler to give patients the same feel of their ongoing salbutamol treatment (Cripps et al., 2000). This approach was extended to salmeterol xinafoate (Seretide Evohaler) (Peyron et al., 2005) and fluticasone propionate (Flixotide Evohaler) (Gabrio et al., 1999). The second approach metamorphosed salbutamol CFC MDI into Airomir (Ross and Gabrio, 1999; Gabrio et al., 1999). Nevertheless, during this transition to HFA MDIs, the aim was to provide similar drug delivery and demonstrate their comparability in efficacy and safety with their predecessor CFC MDIs in simple clinical trials to limit expenses (Cipolla et al., 2010).

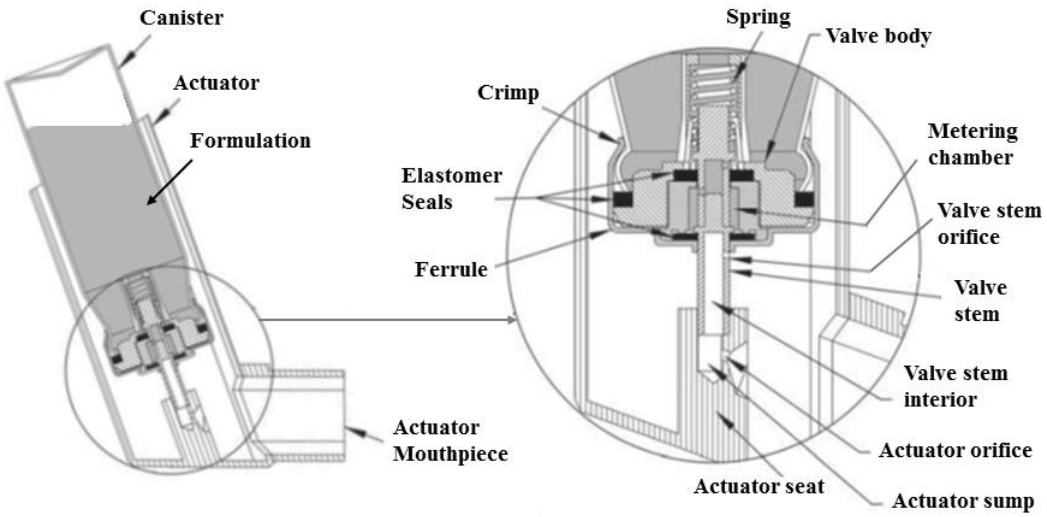


Figure 2.2.1. Schematics of an MDI.
Adapted from Ivey et al. (2014).

Table 2.2.1. Formulation and device design of salbutamol HFA MDIs.

Modifications	Ventolin Evohaler (Ventolin HFA)	Airomir (Proventil HFA)	ProAir, Salamol (HFA)
Formulation			
HFA 134a	Yes	Yes	Yes
Ethanol	No	Yes	Yes
Oleic Acid	No	Yes	No
Device Design			
Actuator Mouthpiece	Rectangular (oval)	Round	Rectangular (oval)
Actuator orifice diameter	0.50 mm[a]	0.25 mm[b]	-
Metered dose volume	63 µL[a]	25 µL[c]	25 µL[d]

[a] Brambilla et al., 2011; [b] Cheng et al., 2001 and Kunda et al., 2017; [c] Ross and Gabrio, 1999; [d] Salamol PIL (Teva, 2015)

2.3 Aerosol Particle Deposition Dynamics and Clinical Effects-ACI Vs HRT

Drug delivery characteristics of an MDI are assessed with cascade impactors which include widely recommended ACI, NGI and Multi-Stage Liquid Impinger (MSLI) (BP, 2005; USP28-NF23, 2005; Ph. Eur., 2011). ACI (Mark II) consists of a stack of eight stages attached to an Induction Port and mimics human respiratory tract (HRT) (Figure 2.3.1). Each succeeding stage has increasing number of nozzle jets with progressively decreasing diameter, and a collection plate underneath (and the back-up filter to the last stage). When an aerosol is drawn with the air through the equipment (Figure 2.3.2), the impaction of progressively smaller particles occurs in the succeeding stages due to differences in inertia - a function of particle aerodynamic diameter and velocity. The velocity increases as particles travel through the impactor resulting in increased particle inertia (Mitchell and Nagel, 2003). At an airflow rate of 28.3 L/min, the particle fractionation ranges from >10.0 to 0.4 µm diameter. Particles <0.4 µm are collected on the backup filter. The impactor separates the discharged dose from an inhaler (the total emitted dose (TED)) into defined size fractions deposited onto individual stages, which are then quantified to generate Aerodynamic Particle Size Distribution (APSD) profile. The APSD is characterised by attributes such as mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) (Newman and Chan, 2008; Stein et al., 2014; Myrdal et al., 2014). Within APSD, the Fine Particle Dose (FPD) consists of particle having aerodynamic diameter <5 µm (EMA, 2006). TED, APSD and FPD of an MDI are its Critical Quality Attributes (CQA) (ICH-Q8(R2), 2009) and key factors affecting its functionality (Sandell and Mitchell, 2015).

Researchers have shown that aerodynamic particle diameter determines deposition in the HRT (Hickey et al., 1996; Howarth, 2001; Pritchard, 2001; Mobley and Hochhaus, 2001; Heyder, 2004; Newman and Chan, 2008). APSD governs pulmonary drug deposition (Darquenne, 2012) and the dose deposited in lungs has been found to be correlated to clinical effects (Laube, 1996; Newman, 1998, 2000). FPD represents the amount of the drug that is considered respirable (Chrystyn et al., 2015). Both FPD and APSD are critical *in-vitro* performance metrics (Stein et al., 2014; Myrdal et al., 2014) that are linked to the efficacy and safety (Clark and Lipworth, 1996a & b; Lipworth, 1996; Newman, 1998, 2000; Newman et al., 2000; Weda et al., 2002 & 2004; Usmani et al., 2003 & 2005; Usmani, 2008; Moore et al., 2017). These studies emphasize that

aerodynamic particle size appropriately describes particle dynamics within the HRT.

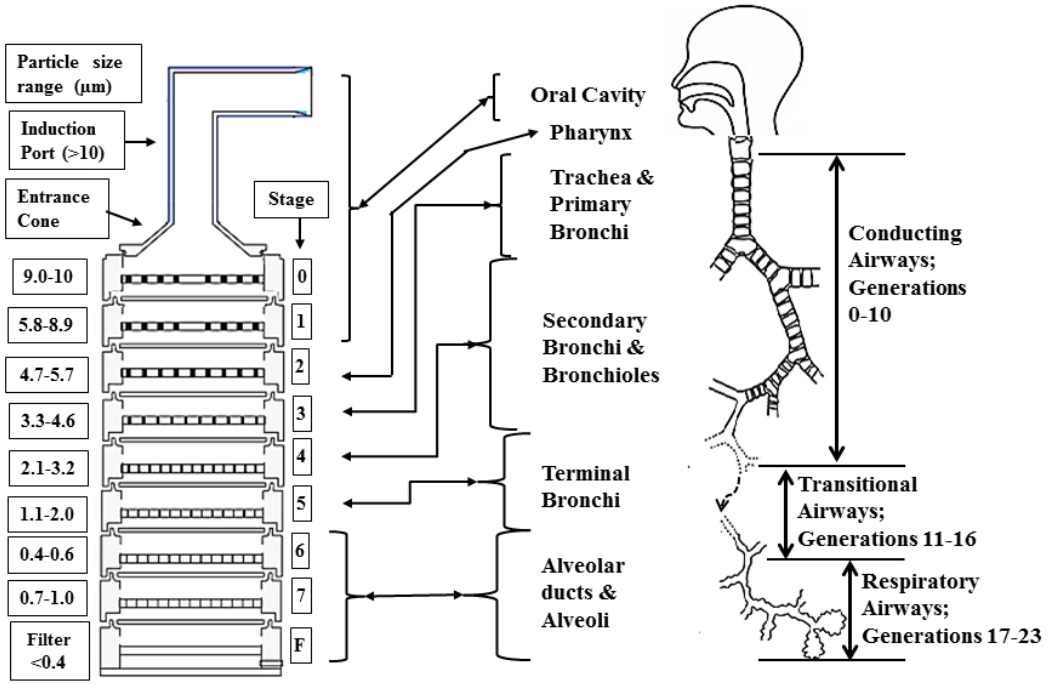


Figure 2.3.1. Schematic comparison of ACI and Human Respiratory Tract.

Adapted and modified from: ACI user manual 1985; Rudolf et al., 1994; Gulak et al., 2009; Ph. Eur. 7.0, 2010; Hussain et al., 2011; Cheng, 2014; https://basicmedicalkey.com/pulmonary/. Accessed 27 Dec 2017.

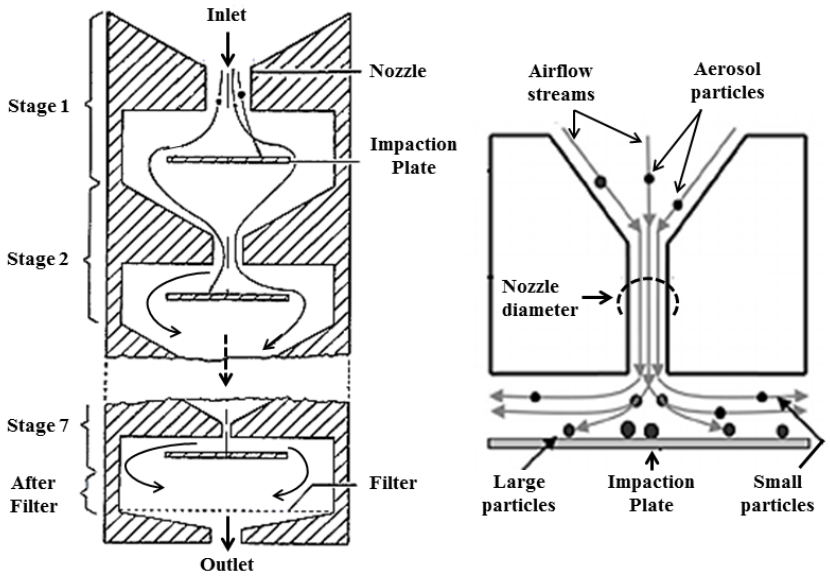


Figure 2.3.2. Schematics of impaction mechanism in ACI.

Adapted and modified from USP 34-NF 29, 2010; Hickey et al., 1996; Hinds, W.C., 1999; Nagao et al., 2005; Mostafa et al., 2015; Elmes and Gasparon, 2017.

The deposition of inhaled drug particles in the HRT takes place by three principal mechanisms (Figure 2.3.3): inertial impaction, gravitational sedimentation and Brownian diffusion (Newman et al., 1982; Schulz, 1998; Hinds, 1999; Zeng et al., 2001; Carvalho et al., 2011; Darquenne, 2012; Tena and Clarà, 2012). Deposition by impaction mainly occurs in the first 10 bronchial generations representing the upper airway (oropharynx, larynx), large, more central, conducting airways and at airway bifurcations (Figure 2.3.1 and Figure 2.3.3). The air velocities are relatively high in these airways and the swift changes in flow direction leads to aerosol particle deposition. Deposition by sedimentation predominates in the last 5 bronchial generations representing the small airways (smaller bronchi and bronchioles) and alveolar regions. Deposition by diffusion primarily takes place in the lung periphery and alveoli, where the airway dimensions are small and air velocities are low. In general, larger drug particles (>10 μm) deposit in the oropharynx, particles of >5 μm in the central airways and particles of 1–5 μm in the small airways and alveoli. Deposition by impaction and sedimentation increases with increasing particle size. In contrast, deposition by Brownian diffusion increases with decreasing particle size and therefore particles <0.5 μm in diameter mainly deposit by this mechanism. Nevertheless, any particles that remain airborne during the respiratory cycle are exhaled, and this occurs most frequently with very small particles (<0.5 μm) (Zeng et al., 2001). However, deposition of inhaled particles can be enhanced by breath holding which is regarded as one of the critical steps of inhalation from an MDI (Section 3.4.4).

Deposition of particles in ACI occurs mainly by inertial impaction under constant airflow (Figure 2.3.2). In HRT (Figure 2.3.3), where the respiratory cycle produces continuously varying airflow, the movement of drug particles in the size range of interest 0.5-10 μm is largely influenced by inertia, to a lesser extent by gravitational sedimentation and least by diffusion (Rudolf et al., 1990).

During inhalation (

Figure 2.3.4), the TED of an inhaler is either deposited into the airways or impacts onto the oropharyngeal region and is swallowed (Chrystyn, 2001; Sakagami, 2006; Laube et al., 2011; Chrystyn et al., 2015). A small fraction of the drug that is deposited into the airways is removed by mucociliary clearance and is also swallowed. The swallowed and inhaled portions of the emitted dose reach the systemic

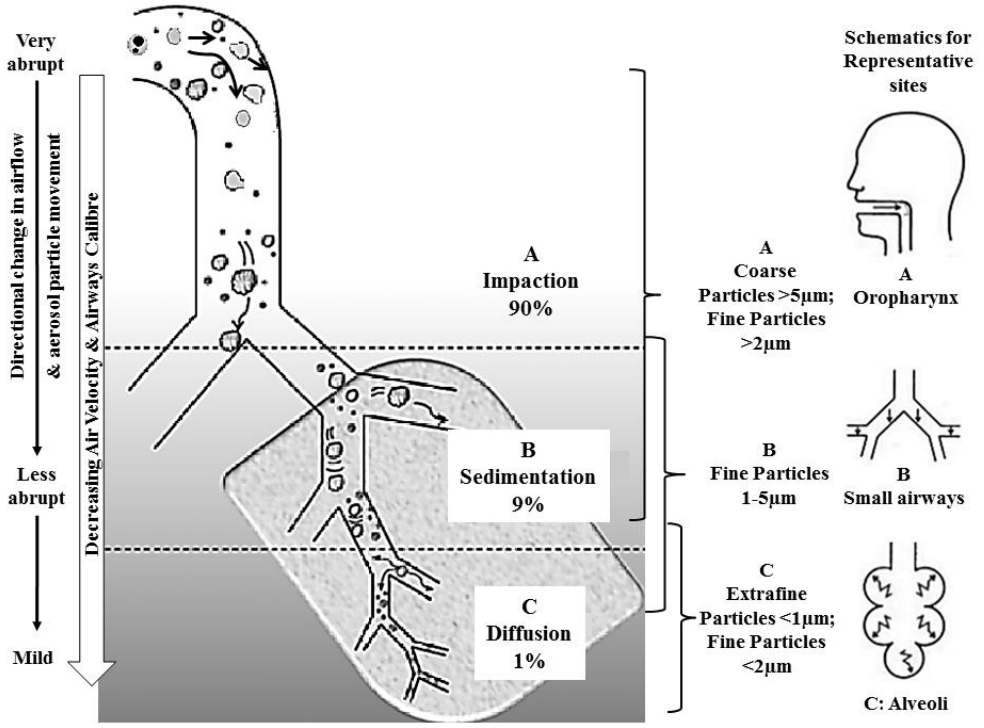


Figure 2.3.3. Schematics of inhaled drug deposition mechanisms in HRT.

Adapted and modified from: Chrystyn, 1999; Carvalho et al., 2011; Hussain et al., 2011; Demoly et al., 2014; http://bronchiectasis.com.au/wp-content/uploads/2015/09/Particle-size.png. Accessed 03 Jan 2018.

circulation through the gastrointestinal and pulmonary routes, respectively. The APSD of the drug, measured *in-vitro* with a cascade impactor, provides an insight into the amount of drug that will impact onto the oropharyngeal region and the distribution of the inhaled fraction in the lungs which includes the FPD. Thus, the information obtained from the APSD profile may predict the likely deposition of the drug particles in the HRT (Newman, 1998). The TED, being a surrogate marker for systemic delivery is therefore considered an indicator of systemic safety, while the FPD and its distribution depict lung deposition, hence regarded as a marker for efficacy (Labiris and Dolovich, 2003; Tena and Clarà, 2012; Chrystyn et al., 2015). Thus, *in-vitro* data can be helpful in estimating the efficacy and safety of inhaled drugs (Olsson et al., 1996; Newman, 2000; Howarth, 2001; Weda et al., 2004; Usmani et al., 2003 & 2005; Usmani, 2008). Further, these *in-vitro* metrics have also been shown to predict pharmacokinetic (PK) and pharmacodynamics (PD) outcomes (Hindle and Chrystyn, 1992; Chrystyn et al., 1998; Chrytyn, 2001; Tomlinson et al., 2003; Mazhar et al., 2008; Mazhar and Chrystyn, 2008; Abdulrahim et al., 2011; Moore et al., 2017). Hence, both *in-vitro* and *in-vivo* studies have been carried out in this project to investigate the presence or absence of this link between them for salbutamol HFA MDIs.

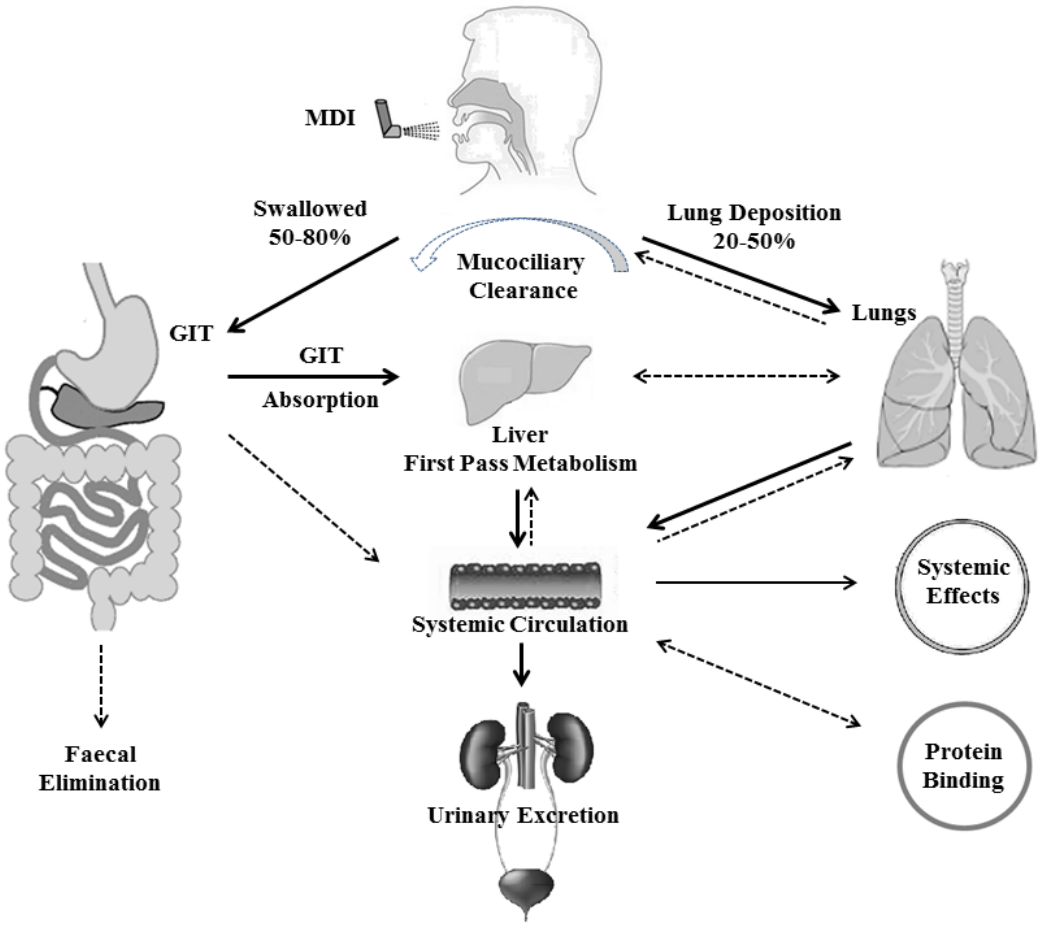


Figure 2.3.4. Fate of inhaled drugs in humans.

Adapted and modified from: Newman, 2000; Chrystyn, 2001; Derendorf et al., 2006; Hochhaus et al., 2015; de Pablo et al., 2017.

2.4 Spacer (Valved Holding Chamber)

Spacer is a chambered add-on device for an MDI which receives the emitted dose to be inhaled (Figure 2.4.1). Spacer use helps patient overcome problems of poor coordination between actuation and inhalation (Lavorini and Fontana, 2009; Dolovich and Dhand, 2011; Nikander et al., 2014). It also retains the ballistic portion of the emitted dose containing large non-respirable particles (>5 µm) thereby significantly reducing the oropharyngeal deposition and local side-effects. The total amount of drug that reaches the patient is also reduced.

Spacers are available in different sizes, volumes, shapes and designs with varying construction material (Hess, 2008; Nikander et al., 2014) and therefore differ in their

drug delivery characteristics (Barry and O'Callaghan, 1995a, 1996 & 1997; Newman, 2004). Large volume spacers provide for greater lung deposition than those with small volumes which may affect the amount of drug available for inhalation (Newman and Newhouse, 1996; Barry and O'Callaghan, 1996; Lipworth and Clark, 1998). However, this varies with drug type and characteristics (Barry and O'Callaghan, 1996).

Even though spacer allows for few seconds to inhale the discharged dose into it (Dolovich et al., 2000), however, it has also been shown that inhalation should take place immediately (Barry et al., 1993). This is because the delayed inhalation may cause the emitted particles to deposit on spacer walls under gravitation and/or electrostatic pull (Wildhaber et al., 1996a; Newman, 2004; Rau, 2006; Rau et al., 2006; Lavorini and Fontana, 2009; Slator et al., 2014; Nikander et al., 2014). If not inhaled immediately, the suspended emitted dose particles remain susceptible to the gravitation force and are also under sustained electrostatic attraction from the spacer walls. Study by Clark and Lipworth (1996a) further substantiates this wherein they observed a two-fold decrease in plasma levels of salbutamol (Ventolin CFC) in healthy subjects following a delay in inhalation compared to no delay. Moreover, patient information leaflets (PIL) of salbutamol MDIs recommend simultaneous press and breathe manoeuvres for inhaling the puff. Further, the inhalation technique using a spacer can only be simulated to MDI alone if the dose discharged in the spacer is immediately inhaled as per PIL recommendations (also see Section 3.4.3). Hence, in this project, no inhalation delay methodology has been applied for *in-vitro* and *in-vivo* studies. On the same grounds, tidal breathing and breathing simulation profile through spacer has not been considered here.

2.4.1 Spacer Electrostatic Charge

Studies have shown that electrostatic charge in a spacer can affect the delivered dose of an MDI (Wildhaber et al., 1996b; Clark and Lipworth, 1997), which is pronounced if inhalation is delayed (Barry and O'Callaghan, 1995b; Clark and Lipworth, 1996a; Wildhaber et al., 1996a & b; Rau et al., 2006). This electrostatic charge in the spacer can be reduced by priming shots of the placebo or drug (Table 2.4.1, Figure 2.4.1), or by washing with ionic detergent aqueous solution. However, differences exist whether the spacer should be rinsed with water before drip-drying (Table 2.4.2; Option I) or not (Table 2.4.3; Option II). These respective differences are also reflected in the spacer

manufacturer's PILs for Volumatic (GSK, 2018) and AeroChamber Plus (TMI, 2008; Blake et al., 2012; Dissanayake et al., 2018). Further, the approach of regional National Health Service (NHS) also differs; for example, NHS Greater Glasgow and Clyde (2013) and NHS North Tees and Hartlepool (2017) recommend final rinsing with water while NHS Cambridgeshire and Peterborough (2016) and NHS University College London Hospitals (2011) do not suggest this step.

Intriguingly, differences in PIL also exist between the Continents and countries. In USA, the FDA requires manufacturers of spacers to recommend that patients rinse them in clean water after washing in detergent, to avoid patient contact with detergent-coated surfaces, which could result in contact dermatitis (Mitchell and Nagel, 2007; Mitchell et al., 2007a; Hess, 2008; Dolovich and Dhand, 2011). This is evident when Rau et al. (2006) highlighted that they followed the manufacturer's United States instructions to pre-treat the seven spacers (including anti-static AeroChamber Max) used in their *in-vitro* study. Kelly et al. (2001) also rinsed AeroChamber Plus with water after detergent treatment as per the USA manufacturer's PIL. These findings re-affirm the existence of different regional approaches for spacer pre-treatment.

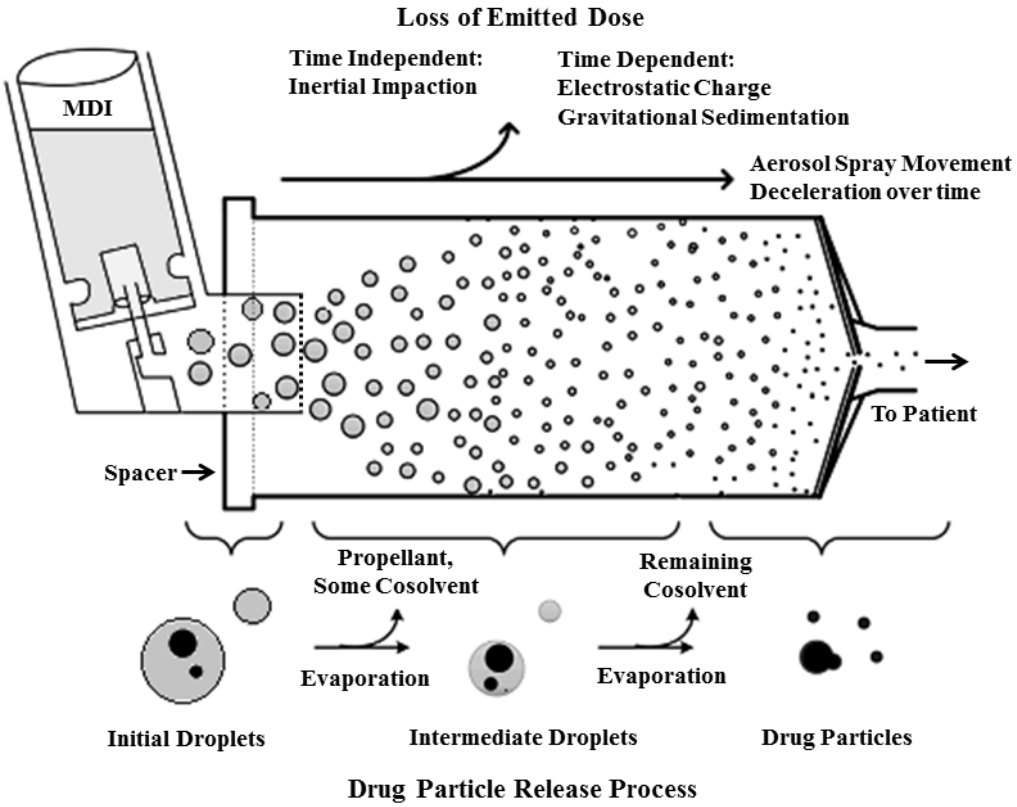


Figure 2.4.1. Schematics of emitted dose aerodynamics of an MDI attached to a spacer. Adapted from Sheth et al., 2014, 2015 & 2017.

Table 2.4.1. List of studies using various options for priming spacers.

Investigators	Methodology	MDI	Spacer	Spacer Treatment
Barry and O'Callaghan, 1995b	MSLI	Budesonide*	Nebuhaler	Anti-static lining ⁘
Clark and Lipworth, 1996a	Plasma PK Study, healthy volunteers (mean age 20.5 years), n=10	Salbutamol (Ventolin) CFC	Volumatic (VOL)	Two VOL coated with antistatic wash resistant polyurethane dispersion⁘. All VOL spacers washed in warm water and left to drip dry.
Barry and O'Callaghan, 1997	MSLI	Ventolin CFC, Airomir	AeroChamber, Nebuhaler	Nebuhaler coated with static dissipative paint (U-100)⁘
Kenyon et al., 1998	MSLI, scintigraphy in asthmatic patients (19–66 years), n=10	Budesonide* (radio labelled)	Volumatic, Nebuhaler, Nebuchamber⚓	20 placebo doses
Fowler et al., 2001	Plasma PK Study	Salbutamol HFA (Airomir)	AeroChamber, cardboard tube	Detergent drip drying and 50 puffs of the MDI
Lipworth et al., 2002	Plasma PK, stable mild asthmatic children (5-12 yr), n=25 (out-patient)	Salbutamol HFA (Ventolin Evohaler)	Volumatic	Benzalkonium chloride (0.05%) coating and drip drying
Land et al., 2014	DUSA	Ventolin Evohaler	Volumatic	20 doses of Ventolin (HFA)

MSLI = Multi Stage Liquid Impinger; DUSA = Dosage Unit Sampling Apparatus; PK = Pharmacokinetic; * Pulmicort; ⚓ Metallic; ⁘ Static Safe Ltd, UK.

Table 2.4.2. List of studies using detergent coating of spacers with option I: Wash with soapy water, rinse with water and drip dry.

Investigators	**Methodology**	**MDI**	**Spacer**
Dewsbury et al., 1996	MSLI	Salbutamol CFC (Salbulin)	Volumatic
Finlay et al., 1997	ACI (delay: 1 sec)	Ventolin CFC and Beclomethasone (Beclovent)	Space-Chamber, AeroChamber
Finlay and Zuberbuhler, 1999	ACI (tidal breathing)	Airomir	AeroChamber, OptiChamber, E-Z Spacer, Vent170, NES spacer[†⚓]
Silkstone et al., 2002a	ACI	Ventolin CFC	Volumatic
Rau et al., 2006	ACI (delay: 2 and 5 sec)	Ventolin HFA	AeroChamber Max, Vortex (metallic), OptiChamber Advantage, ProChamber, Breathrite, PocketChamber, ACE[‡]
Goncalves et al., 2013	NGI	Beclomethasone+Formoterol[⁂]	Able Spacer, AeroChamber Plus, Vortex[⚓]
Hatley et al., 2014	NGI (flow rates: 15 and 30 L/min)	Salbutamol HFA (ProAir), Beclomethasone (QVAR)	AeroChamber Z-Stat, AeroChamber Plus, OptiChamber Diamond
Slator et al., 2014	Collected on filter; (delay: 0, 5, and 10 sec; flow rates: 5, 15, and 30 L/min)	ProAir	AeroChamber Z-Stat, AeroChamber Plus, OptiChamber Diamond

ACI = Andersen Cascade Impactor; NGI = Next Generation Impactor; [†] Nebuchamber; [‡] Aerosol Cloud Enhancer; [⁂] Innovair
Other notations explained as for (Table 2.4.1).

Table 2.4.3. List of studies using detergent coating of spacers with option II: Wash with soapy water and drip dry; no rinse with water.

Investigators	**Methodology**	**MDI**	**Spacer**
Wildhaber et al., 1996a	MSLI; (also with delay: 1, 5 and 20 sec in two spacers)**	Ventolin CFC	Babyhaler**, Nebuchamber**⚓, Babyspacer, AeroChamber, Nebuhaler
Wildhaber et al., 1996b	MSLI	Ventolin CFC	Volumatic
Piérart et al., 1999	MSLI and healthy volunteers (25–42 years), n=8	Ventolin CFC (radio labelled)	Volumatic
Anhøj et al., 1999	Plasma PK Study, children (7–12 years), n=5	Salbutamol HFA (Sultanol)	Babyhaler, AeroChamber
Mitchell et al., 1999	ACI	Ventolin CFC, Airomir	Volumatic
Wildhaber et al., 2000a	PD Study, FEV_1, asthmatic adults (18-65 years), n=20	Ventolin CFC	AeroChamber, Volumatic
Wildhaber et al., 2000b	MSLI, Radioactivity measurement, stable asthmatic children of age <48 (n=8)^ months and >48 months (n=10)	Ventolin CFC (radio-labelled)	^Babyhaler (with mask), Volumatic
Dompeling et al., 2001	PD Study, PEF, asthmatic children (4–8 years), n=90	Ventolin HFA	AeroChamber, Volumatic, Nebuchamber⚓
Chuffart et al., 2001	MSLI, PD Study, FEV_1, asthmatic children (13–17 years), n=12	Ventolin CFC, Airomir	AeroChamber, Nebuhaler, Volumatic
Barben et al., 2003	PD Study, FEV_1, stable asthmatic children (7–18 years), n=50	Ventolin HFA	Volumatic
Dubus et al., 2003	PD Study, Methacholine challenge, FEV_1, asthmatic young children (3-6 years), n=64	Ventolin HFA	Babyhaler (static and non-static) and Nebuchamber⚓, both with their own facemasks.

Notations explained in Table 2.4.2 and Table 2.4.3.

Interestingly, the clinical evidence is conflicting with regards to spacers that were treated with detergent only (Table 2.4.3). Anhøj et al. (1999) observed that the plasma concentration of salbutamol HFA (Sultanol®) in children increased by over two-fold with non-electrostatic Babyhaler as compared to non-conducting Babyhaler and AeroChamber. Wildhaber et al. (2000a) recorded improved bronchodilator response (FEV_1) to salbutamol (Ventolin CFC) in asthmatic adults with detergent treated Volumatic. Wildhaber and colleagues (2000b) also found higher lung deposition of radio-labelled salbutamol (Ventolin CFC) in children using Babyhaler (with mask) and Volumatic. Moreover, Chuffart et al. (2001) reported increase in FEV_1 at 5 min post-inhalation in asthmatic children when salbutamol HFA (Airomir) was used with non-static Nebuhaler as compared to static Nebuhaler attached to Ventolin CFC. In contrast, Clark and Lipworth (1996a) found significant increase in C_{max} of Ventolin CFC inhaled by healthy adults from the untreated Volumatic compared with the antistatic treated Volumatic (both prewashed with warm water only) and no other differences were seen in average plasma salbutamol levels (C_{ave}) or systemic β_2 responses (Table 2.4.1). They concluded that washing a Volumatic with water was as effective as coating it with an antistatic spray in reducing the effects of static charge on salbutamol delivery *in-vivo*. Besides, Dompeling et al. (2001) could not observe significant differences in peak expiratory flow rate (PEF) of asthmatic children when they inhaled salbutamol (Ventolin HFA) with Volumatic and AeroChamber (static and non-static), and Nebuchamber (metallic) (Table 2.4.3). They concluded that electrostatic charge on plastic spacers did not decrease the efficacy of bronchodilator therapy in their subjects. These observations are also supported by Barben et al. (2003) who could not find a clinical benefit of detergent-coated Volumatic after a single dose of salbutamol (Ventolin HFA) in asthmatic children. There was no difference in FEV_1 and maximal mid-expiratory flow (MMEF) at 10 and 20 min after inhalation from either coated or non-coated Volumatic. However, they noticed a small statistically significant difference in PEF at 10 min, which disappeared after 20 min. Further, Dubus et al. (2003) also found no difference in methacholine challenged bronchodilation with salbutamol (Ventolin HFA) administered to asthmatic young children using Babyhaler (static and non-static) and Nebuchamber, all equipped with their own facemasks.

Nevertheless, it is noted that these studies have shortcomings. Wildhaber et al. (2000b) did not provide any data for static spacers to show the actual difference in lung

deposition of radio-labelled salbutamol in children. Chuffart et al. (2001) measured FEV_1 at 5 min while EMA (2009) requires this be measured at 15 min. Further, there is an inherent bias in this study as two different delivery systems have been compared with (Airomir + non-static Nebuhaler Vs Ventolin CFC + static Nebuhaler). Consequently, the results may have been affected by a number of varied factors originating from differently formulated MDIs (Table 2.2.1) and differently treated spacers. The results therefore cannot be reliably linked to the potential benefit of detergent treatment of spacer. Discussing their apparently out of trend results, Dompeling et al. (2001) explained that the difference between coated and non-coated spacer may have been masked by either a good inhalation technique of children or the dosage used may already have been at the higher part of the dose-response curve. However, these researchers used a different outcome measure PEF and therefore their results may not be comparable to studies using FEV_1. Nevertheless, Barben et al. (2003) used FEV_1, PEF and MMEF as outcome measures and reached the same conclusions as those of Dompeling and co-workers. It is interesting to note that in study by Dompeling et al., the two non-conducting plastic spacers were considered electrostatic after washing these with water only and this was shown with the significant difference in their measured electrostatic charge. However, Clark and Lipworth (1996a) also pre-washed both static and anti-static spacers with warm water. Although they did not measure the electrostatic charge on Volumatic, their conclusions are in agreement to those of Dompeling and colleagues. Intriguingly, the data of *in-vitro* study by Dubus et al. (2001) shows similar FPD (<5.8 µm) of Airomir from non-conducting (AeroChamber-Infant and Babyhaler) and conducting (NebuChamber, metallic) spacers, all of which were washed with lukewarm water only (Table 2.5.2). Their reported FPD of Airomir with AeroChamber-Infant (68 µg) is also similar to that of Mitchell et al. (1999) (62 µg) for this combination, the apparent difference in FPD is more likely due to the latter's FPD being <4.7 µm. This is despite Mitchell and co-workers prewashed and drip dried the spacer with ionic detergent to minimize the influence of electrostatic charge. These contradicting *in-vitro* findings raise questions and may suggest that spacer preparation method may not be of significance or perhaps irrelevant in this case.

Further, it can be noticed that most of these clinical studies were performed in children except those of Clark and Lipworth (1996a) and Wildhaber et al. (2000a). However, the former used Ventolin CFC while it is not clear if Ventolin HFA was used in the latter

study. Nevertheless, Anhøj et al. (2000) found that lung deposition (plasma concentration) of inhaled budesonide (Pulmicort) administered with Nebuchamber increased with age in mild stable asthmatics aged 2–3 and 4–6 year and adults older than 18 year. It is also known that children have different breathing patterns, respiratory anatomies and capacities, volume of distribution and clinical response than those of adults (Labiris and Dolovich, 2003; Rubin and Fink, 2005; Venegas et al., 2013). Therefore, extrapolating the results of one age group to a different one is difficult to justify (EMA, 2009). Also, experiments with one spacer (similarly or differently treated) or a specific drug cannot be extrapolated to others (Barry and O' Calaghan, 1997; Liworth and Clark, 1998). In addition, studies using different methodologies, outcome measures, subjects (patients or healthy), ages, types and dosages are difficult and inappropriate to compare. Further, given that the clinical evidence in children is conflicting, and that the clinical evidence in adults is scarce, the effects of rinsing or not rinsing spacers with water after detergent treatment on salbutamol dose delivery efficiency remains obscure (Lavorini and Fontana, 2009; Laube et al., 2011).

The foregoing literature review reveals lack of consensus on whether spacers should be rinsed after cleaning with detergent. Besides, the PK and PD evidence is conflicting and insufficient for HFA salbutamol MDI delivery with these spacers. Hence, the conclusion drawn from salbutamol CFC MDIs studies cannot be representative of those of HFA MDIs (Liu et al., 2017) due to differences in their formulation and device design (Table 2.2.1) and where different spacers were used. Therefore, based on the information extracted from the literature, PILs and FDA recommendations, the spacers used in this project have been treated with aqueous detergent solution followed by water rinsing and drip-drying (Section 3.3.2).

2.5 *In-Vitro* Particle Size Characterisation Studies

A summary of *in-vitro* studies for Ventolin Evohaler, Airomir and ProAir is given in Table 2.5.1, Table 2.5.2 and Table 2.5.3, respectively. The studies conducted during transition from CFC to HFA propellant were carried out either by pharmaceutical companies themselves or sponsored by them. Parallel *in-vitro* studies were also carried out by the manufacturers of spacers which are ongoing with the introduction of newer MDIs and/or spacers.

These *in-vitro* studies were carried out using Andersen Cascade Impactor (ACI) with a USP induction port operated at a flow rate of 28.3 L/min. When Next Generation

Impactor (NGI) was in use, this was operated at 30 L/min. Airflow duration varied between studies from 8 to 30 seconds while in one study, it was timed to allow 4 L of air to pass through the instrument (McCabe et al., 2012). In general, 5 to 10 puffs of the MDI were used for each experiment (with 3 to 6 replicates), even though the clinically relevant dose of salbutamol MDI is upto 2 puffs (BNF, 2017). In most studies, ACI impaction plates or NGI impaction cups were not coated. Where spacers were used with the MDI, some researchers chose to pre-treat these with a detergent while others did not; also pre-treatment procedure varied between them as discussed earlier (Section 2.4). Further, one study did not provide spacer identity (Ross and Gabrio, 1999). APSD data analysis and reporting of full profiles was selective and not all studies provided data on TED (and TDD ex-spacer), FPD, MMAD and GSD. Statistical comparisons were not provided in some studies (Ross and Gabrio, 1999; Cripps et al., 2000). The ambiguity in the experimental details, the lack of availability of essential MDI performance data (including stage- or stage-group wise comparisons) and the use of clinically relevant doses represent the main areas where improvements are needed in future studies. A review of full and detailed publications on *in-vitro* studies follows.

Ross and Gabrio (1999) described the development of Airomir (Table 2.5.2). These researchers compared Airomir with Ventolin CFC used alone and with unspecified small and large volume spacers using ACI. Based on the results, they suggested that FPD (<4.7 µm), fine particle fraction (FPF), MMAD and Induction Port (IP) (throat) deposition of the two MDIs with these spacers was comparable and that these spacers had similar effects on both MDIs. However, these claims were not substantiated with the statistical evidence. Their data shows an increase in FPD with the spacers as compared to MDI alone and the differences in the magnitude of this increase by the two unspecified spacers were not highlighted. Interestingly, a closer look at their data for Airomir shows that FPDs obtained with small and large spacers are greater than MDI alone, having ratios of 1.30 and 1.56, respectively. Further, it is also not clear how many puffs were used (although 20 puffs were used in their temperature cycling study on these MDIs), whether ACI plates were coated to prevent the particle bounce and re-entrainment (Nasr and Allgire, 1995; Nasr et al., 1997; Kamiya et al., 2004), and what was the airflow duration. Also, APSD graphic profile in their manuscript does not show salbutamol deposition on the filter stage and no explanation was provided for this omission. Further, it is not elaborated whether spacers were neutralised for electrostatic charge.

Cripps et al. (2000) reported the seamless pharmaceutical transition of Ventolin CFC to Ventolin Evohaler (Table 2.5.1). These researchers used ACI fitted with a small volume metal induction port (IP) which is likely to have different dimensions and volume than the IP of USP/Ph. Eur. It was concluded from the results that particle size distribution profiles of the two MDIs were similar, albeit without providing the statistical evidence.

Comparing Ventolin HFA MDI alone and when attached to Babyhaler and Volumatic, Cripps and co-workers concluded that spacers did not significantly affect APSD; again without supporting the claim with statistical data. However, assessment of their available data reveals that FPD of Ventolin HFA with Volumatic is considerably higher (ratio) than both MDI alone (1.27) and with Babyhaler (1.30). These researchers did not coat the ACI plates. Hence, the reported particle bounce and re-entrainment on uncoated plates (Nasr and Allgire, 1995; Nasr et al., 1997; Kamiya et al., 2004) was evident from a high capture of particles on the filter. Also, it is not clear if the spacers were pre-treated to minimise electrostatic charge. Further, these researchers defined FPD to consist of deposition on stages 2 to 6 of ACI.

Mitchell et al. (1999) compared Ventolin CFC and Airomir without and with AeroChamber and Volumatic (Table 2.5.2). Spacers were treated with detergent and drip dried. ACI was stacked with glass collection plates. Their data shows a greater magnitude of increase in FPD of Ventolin CFC when used with Volumatic than AeroChamber, albeit both FPDs being greater than that of the MDI alone. They also observed significantly larger FPD of Airomir with these spacers, however, the differences of FPD between these spacers were smaller even though approaching statistical significance ($p = 0.056$). They inferred from these results that increase in spacer volume between ~140 and 750 mL may have only small impact on FPD of salbutamol HFA MDIs. However, this inference should have been qualified to Airomir only since Hall et al. (2011) have reported an increased FPD of Ventolin HFA with Volumatic than Breath-a-Tech with a ratio of 1.29. Although Volumatic is not recommended for use with Airomir, it is not clear as to how Mitchell and co-workers connected the round actuator mouth piece was connected to the roughly rectangular (oval) inhaler port of the spacer. If a rectangular shaped actuator was substituted for Airomir canister (as shown in the manuscript), it can have implications for TED and the resultant APSD profile may not be representative of the original Airomir actuator. On the other hand, if the inhaler port of Volumatic was changed to a round hole, it can also

influence APSD profile besides this being impractical and irrelevant. Further, these investigators estimated MMAD from the log normal plots of cumulative mass % less than stated size for both spacer combinations. In contrast, MMAD for MDI alone was deduced by separating the IP depositions from the impactor mass. This approach was adopted since the cumulative mass % less than stated size could not exceed 50% cut-off point; a plateau was observed at ~40% for Ventolin CFC MDI and at ~50% for Airomir. Nevertheless, the reported MMAD of >9 µm remains questionable.

Mitchell and co-workers also reported %FPF of >96% for both MDIs and spacer combinations and suggested their similar FPD delivery efficiency (Table 2.5.2). However, FPD for Ventolin CFC with AeroChamber and Volumatic was 45.4 µg and 63.8 µg, respectively, which is significantly different. Their respective TED was 47.2 µg and 66.1 µg. On the other hand, FPD of Airomir with these spacers was 62.0 µg and 67.9 µg, respectively, which are comparable. Their respective TED was 64.2 µg and 69.7 µg. It is clear that for both MDIs, FPD was conspicuously larger with Volumatic than AeroChamber. However, because this magnitude of increase was not observed with their TEDs, %FPF was similar thereby suggesting their apparent similarity in dose delivery efficiency. Although the scale of error of using the derived data (%FPF) reported in this study is more with Ventolin CFC than Airomir, the use of this outcome measure has high potential of misleading and jeopardising objective decision making (also see Section 6.2.7.5).

Dubus et al. (2001) reported particle size characterisation of four salbutamol MDIs (Airomir, Ventolin CFC and two French generics) used alone and with three spacers (AeroChamber-Infant, Babyhaler and metallic NebuChamber) (Table 2.5.2). These spacers were treated by washing with lukewarm water only. ACI impaction plates were used without coating. Higher FPD (<5.8 µm) is reported for Airomir than Ventolin CFC (ratio 1.34). FPD of both MDIs increased with spacers as compared to MDI alone, however, the magnitude of this increase for Ventolin CFC was lesser with AeroChamber than the other two spacers. The FPD of Airomir obtained from the conducting NebuChamber was only slightly larger (ratio) than non-conducting Babyhaler (1.08) and AeroChamber (1.07) while it was similar between the non-conducting spacers (0.99). The corresponding FPD ratios for Ventolin CFC obtained from these spacers are 1.03, 1.27 and 1.23. Further, FPD with spacers reflected on their respective delivered dose for both MDIs. Although more salbutamol dose was delivered from NebuChamber as compared to the other two spacers, the data suggest that the

formulation and plume ballistic characteristics of the two MDIs, along with the spacer volume, had more contribution to this than the conducting material or the spacer washing method. It is also noted that %FPF was calculated with TED obtained from Emitted Dose Uniformity (EDU) tests performed separately rather than the TED obtained from same ACI data. MMAD and GSD for MDI alone were not provided to assess the impact of spacer on them.

Johnson et al. (2016) compared APSD of Ventolin HFA, Proventil HFA and ProAir HFA alone and with LiteAire spacer. The salbutamol amounts have been reported as sulphate (see base equivalent conversions underneath Table 2.5.1-Table 2.5.3). The FPD of Ventolin HFA MDI alone is atypically very small while IP deposition is large as compared to other inhalers. The TED for MDI alone meets mass balance requirement (±25% of the labelled claim) for ProAir HFA only. Spacer deposition could not be reported due to its cardboard construction. With spacer, FPD for Ventolin HFA and Proventil HFA increased while it decreased for ProAir. The TDD and FPD of Ventolin HFA with LiteAire are significantly lower than those reported by Hall et al. (2011). Also, there seems to be issues with the sensitivity of the analytical method as some stage depositions could not be quantified.

McCabe et al. (2012) performed comparative APSD of Ventolin HFA and ProAir HFA using NGI at a flow rate of 28.3 L/min and flow volume of 4 L (~8.5 seconds) (Table 2.5.1 and Table 2.5.3). The flow rate used in their study is not pharmacopoeia recommended. The NGI collection cups were not coated. Their reported FPD for Ventolin HFA is out of trend.

Hall et al. (2011) have reported spacer only studies with Ventolin HFA and therefore cannot be related to MDI alone performance. They have reported an insignificant increase in TDD from large-volume (>500 mL) than small-volume (<250 mL) spacers. The TDD was averaged for these two groups of three spacers each (Table 2.5.1). Their data does not reveal any linking trend for spacer volume and shape to TDD and FPD. Although these metrics are atypically low and out of trend for Space Chamber, the %FPF suggests similarity of dose delivery efficiency to other spacers which is grossly erroneous as discussed above.

Table 2.5.1. *In-vitro* studies on Ventolin HFA without and with spacers.

Ventolin MDI or MDI+SP[1]	**TED / TDD[2]**	**SP[3]**	**SP[3] Vol (mL)**	**SP[3] Treatment**	**IP[4]**	**FPD[5]**	**FPF[6] (%)**	**MMAD[7] (µm)**	**GSD[8]**	**ACI Plate Coating**	**Flow Time (sec)**	**No. of puffs**	**Reference**
Ventolin[§]	82	N/A	N/A	N/A	39	34[#]	42[#]	2.1	?	No	?	5	Cripps et al., 2000; n=6
+ Volumatic[§]	90 (TED) 53^ (TDD)	37	750	No	1.3	43[#]	48[#] (TED); 82[#] (TDD)^	?	?				
+ Babyhaler[§]	93 (TED) 39^ (TDD)	54	350	No	0.7	33[#]	36[#] (TED); 85[#] (TDD)^	?	?				
+ Volumatic	40.8	?	750	Yes[a]	?	37.2	91.2	?	?	No**	10; 20 sec last puff	10	Hall et al., 2011; n=6
+ Breath-a-Tech	32.8	?	218	Yes[a]	?	28.9	88.2	?	?				
+ Space Pod	30.7	?	800	Yes[a]	?	29.5	96.2	?	?				
+ Space Chamber	16.6	?	225	Yes[a]	?	15.6	93.6	?	?				
+ e-Chamber	37.2	?	510	Yes[a]	?	33.7	90.6	?	?				
+ LiteAire	33.4	?	160	N/A	?	31.3	93.8	?	?				
Ventolin; NGI; 28.3 L/min	85	N/A	N/A	N/A	?	26	31	2.4	2.0	No	4 L	10	McCabe et al., 2012; n=6
Ventolin HFA[‡]	86.5[‡]	N/A	N/A	N/A	61.6[‡]	20.9[‡]	24.2	2.48	1.77	No	30	5	Johnson et al., 2016; salbutamol as sulphate, n = 6
+ LiteAire[‡]	26[‡] (TDD) 91[‡] (TED)^	62[‡]^	160 cc	N/A	2.8[‡]	25[‡]	94 (TDD); 27 (TED)	2.08	1.71				

[1] MDI with spacer; [2] Total Emitted/Delivered Dose; [3] Spacer; [4] Induction Port; [5] Fine Particle Dose; [6] Fine Particle Fraction; [7] Mass Median Aerodynamic Diameter; [8] Geometric Standard Deviation; N/A = Not Applicable; ? = data/information not provided.

[a] Option II: Washed with soapy water and drip dried (Table 2.4.3).

FPD < 5.8 µm (stages 2-6)

* Calculated by this author from the data in the manuscript.

** Personal communication

^ Value estimated by this author from the data in the manuscript.

§ FPD (stages 3-F) and FPF (%) estimated by this author (Cripps et al., 2000): Ventolin FPD (37), FPF% (46); Ventolin+ Volumatic FPD (47), FPF%TED (52), FPF%TDD (~88); Ventolin+ Babyhaler FPD (35), FPF%TED (38), FPF%TDD (~91).

‡ Salbutamol base equivalent (calculated by this author) (Johnson et al., 2016): Ventolin HFA TED (72.2), IP (51), FPD (17.4), FPF% (24.03); Ventolin HFA+ LiteAire TDD 21.8 (ex-SP), TED (75.7) (ex-actuator)^, spacer deposition (51.2)^, IP (2.3), FPD (21).

Table 2.5.2. *In-vitro* studies on Airomir without and with spacers.

Airomir MDI or MDI+SP[1]	**TED / TDD[2]**	**SP[3]**	**SP[3] Vol (mL)**	**SP[3] Treatment**	**IP[4]**	**FPD[5]**	**FPF[6] (%)**	**MMAD[7] (µm)**	**GSD[8]**	**ACI Plate Coating**	**Flow Time (sec)**	**No. of puffs**	**Reference**
Airomir	99.4*	N/A	N/A	N/A	44.0	49.8	50.1	2.23	?	?	?	?	Ross and Gabrio, 1999; n=3
+ small volume SP[1]	74.4* (TDD)	?	?	?	2.9	64.5	86.7	2.50	?			20[C]	
+ large volume SP[1]	89.0* (TDD)	?	?	?	1.9	77.7	87.3	2.67	?				
Airomir	81.3	N/A	N/A	N/A	~43*	38.7	47.6*	> 9	?	? (Glass plates)	30	5	Mitchell et al., 1999; n=3
+ AeroChamber	64.2 (TDD)	?	145	Yes[a]	?	62.0	96.6*	2.6	?				
+ Volumatic	69.7 (TDD)	?	750	Yes[a]	?	67.9	97.4*	2.4	?				
Airomir;	85.3†	N/A	N/A	N/A	?	54.1[֍]	63.5*	?	?	?	30	5	Dubus et al., 2001; n=6
+ AeroChamber	69.9†	?	145	Yes[b]	?	68.3[֍]	97.7*	2.78	1.47				
+ Babyhaler	68.5†	?	350	Yes[b]	?	67.5[֍]	98.5*	2.68	1.39				
+ NES-spacer[⚓]	80.2†	?	150	Yes[b]	?	73.2[֍]	91.3*	2.77	1.47				
Proventil HFA[‡]	75.04[‡]	N/A	N/A	N/A	34.3[‡]	40.1[‡]	53.4[♦]	2.22	1.57	No	30	5	Johnson et al., 2016; salbutamol as sulphate, n = 6
+ LiteAire[‡]	53.8[‡] (TDD) 87.1[‡] (TED)	29.1[‡]^	160 cc	N/A	4.78[‡]	52.5[‡]	98 (TDD); 60 (TED)^	2.26	1.41				

[b] Washed with lukewarm water only. [C] Temperature cycling study.

† TED (TDD) obtained separately from sampling tube experiments (EDU); n=30, Flowrate = 28.3 L/min for 10 seconds.

‡ Salbutamol base equivalent (calculated by this author) (Johnson et al., 2016): Proventil HFA TED (62.3), IP (28.5), FPD (33.3), FPF% (53); Proventil HFA + LiteAire TDD (44.7) (ex-SP), TED (72.3) (ex-actuator)^, spacer deposition (25)^, IP (4.0), FPD (43.6)

♦ (58% in the manuscript; more likely a typo error)

⚓ Nebuchamber

֍ FPD = < 5.8 µm (stages 2-7 and F) (Dubus et al., 2001)

Other notations explained as for Table 2.5.1.

Table 2.5.3. *In-vitro* studies on ProAir HFA without and with spacers.

ProAir MDI or MDI+SP[1]	**TED / TDD[2]**	**SP[3]**	**SP[3] Vol (mL)**	**SP[3] Treatment**	**IP[4]**	**FPD[5]**	**FPF[6] (%)**	**MMAD[7] (µm)**	**GSD[8]**	**ACI Plate Coating**	**Flow Time (sec)**	**No. of puffs**	**Reference**
ProAir; NGI, 28.3 L/min	93	N/A	N/A	N/A	?	53	57	2.3	1.6	No	4 L (~8.5 sec)^	10	McCabe et al., 2012; n=11
ProAir, 90 µg❖	90.7❖	N/A	N/A	N/A	29.7	57.8❖	63.7	2.35	?	No	20 sec (last puff 30 sec) (Hatley et al.)	10	Hatley et al., 2014 and von Hollen et al., 2011a & b, 2012 & 2013; NGI, 30 L/min; MDI n=12, MDI+SP n=6
+ AeroChamber Plus; 90 µg❖	68.5❖ (TDD) 89.2^ (TED)	20.7^	149	Yes[d]	?	58.1❖	84.8 (TDD) 65.1^ (TED)	2.39	?				
+ AeroChamber Plus Z-Stat; 90 µg❖	72.3❖ (TDD) 88.5^ (TED)	16.2^	149[e]	Yes[d]	7.6	61.1❖	84.4 (TDD) 69.0^ (TED	2.40	?				
+ OptiChamber Diamond; 90 µg❖	69.7❖ (TDD) 89.5^ (TED)	19.8^	140	Yes[d]	4.6	61.2❖	87.8 (TDD) 68.4^ (TED	2.41	?				
ProAir‡	106.7‡	N/A	N/A	N/A	38.9‡	64.4‡	61	2.4	1.53	No	30	5	Johnson et al., 2016; salbutamol as sulphate, n = 6
+ LiteAire‡	63.2‡ (TDD) 108.6‡^ (TED)	42.9‡^	184 cc	N/A	3.07‡	61.4‡	97 (TDD); 56.5^ (TED)	2.33	1.48				

[d] Option I: Washed with warm soapy water, rinsed with deionised water and drip dried (Table 2.4.2).

❖ Salbutamol base equivalent rounded to 100 µg (ex-valve dose) (Hatley et al., 2014 and von Hollen et al., 2011a & b, & 2012): ProAir HFA TED (100.8), FPD (64.6); ProAir HFA + AeroChamber Plus TDD (76.1), TED (99.1), spacer deposition (23), FPD (64.6); ProAir HFA + AeroChamber Plus Z STAT TDD (80.3), TED (98.3), spacer deposition (18), FPD (67.9); ProAir HFA + OptiChamber Diamond TDD (77.4), TED (99.4), spacer deposition (22), FPD (68.0).

‡ Salbutamol base equivalent (calculated by this author) (Johnson et al., 2016): ProAir HFA TED (88.6), IP (32.3), FPD (53.7) ProAir HFA + LiteAire TDD (52.5) (ex-SP), TED (90.2) (ex-actuator)^, spacer deposition (35.6)^, IP (2.6), FPD (51.0).

[e] Li et al., 2008

Other notations explained as for Table 2.5.1.

2.6 The effect of Spacer on FPD of Salbutamol HFA MDIs

The data reported by Ross and Gabrio (1999) shows significantly greater ratios of FPD of Airomir when used with unspecified small (1.30) and large volume (1.56) spacers as compared to MDI alone. Mitchell et al. (1999) also reported that FPD of Airomir with both AeroChamber (small volume) (1.60) and Volumatic (large volume) (1.75) was significantly higher than the MDI alone. On the other hand, Cripps et al. (2000) could not find any significant difference in FPD of Ventolin HFA between MDI alone and when attached to Babyhaler (0.97) or Volumatic (1.27), although it was greater with the latter spacer. Studies on ProAir reveal similar FPD with small volume spacers (AeroChamberPlus, AeroChamber Z-stat and OptiChamber Diamond) as compared to the MDI alone (von Hollen et al., 2011a & b, & 2012; Hatley et al., 2014). To the knowledge of this author, no studies have been reported with large volume spacers for ProAir or Salamol.

Formulation and MDI device design influence the dose delivery characteristics (Stein et al., 2014; Myrdal et al., 2014). The differences in salbutamol HFA MDI formulations have been shown to be affected differently with the spacer volume and the magnitude of this effect varied with each type of spacer. Cripps et al. (2000) reported greater FPD (ratio) when Ventolin HFA was attached to Volumatic than Babyhaler (1.30). Hall et al. (2011) also found similar trend between Volumatic and Breath-a-Tech (1.29) for Ventolin HFA. Greater FPD has also been reported by Ross and Gabrio (1999) for unspecified large spacer than the small one (1.21) for Airomir. However, this magnitude of increase in FPD of Airomir was lesser between Volumatic and AeroChamber (1.10) in the study by Mitchell et al. (1999). Interestingly, ProAir HFA has been shown to have similar FPD between the three spacers (von Hollen et al., 2011a & b, & 2012; Hatley et al., 2014).

These findings do not reveal any general trending in FPD of either salbutamol HFA MDI alone and when used with a spacer. Further, this trend was also not clearly evident with the spacers used with these MDIs.

2.7 Summary and Conclusions – Literature Review

The review of *in-vitro* studies reveals high variability in FPD amongst and within each of the three salbutamol HFA MDIs when used alone. This is expected since these HFA MDIs are differently formulated and dispensed with differently designed devices (Table 2.2.1). Further, differences amongst *in-vitro* studies conducted by different investigators for the same MDI are of a common occurrence. This is because APSD measurements using ACI are a very sensitive technique with many potential sources of variability. Moreover, spacers can introduce additional sources of variability, in particular due to their handling technique and experimental set-up. The addition of a spacer to an MDI creates a new drug delivery system which can change APSD profile; the extent of which depends on a number of spacer-related factors. Differences in spacer characteristics (construction material, size, volume and dimensions, valve type and design) coupled with pre-treatment and handling methods (new, water washed, detergent washed without or with water rinsing, antistatic coating, priming) have been reported to have influenced APSD to a varied degree (Table 2.4.1 to Table 2.5.3). Hence, the APSD data would reflect on these differences.

It has been reported that both large and small volume spacers attached to Airomir provide for larger FPD than the MDI alone; the degree of this increase is greater with the large volume spacer. However, difference in FPD of Ventolin Evohaler was identified as not significant with both large and small spacers as compared to MDI alone. Comparative studies on ProAir did not report any difference in FPD obtained with small spacers and MDI alone.

It is also noted that 5 or more MDI puffs were used in all previous *in-vitro* studies while the recommended dose for patients is 1 to 2 puffs. Besides, the cascade impactor experimental procedure has not been reported clearly in most studies and where a pharmacopoeia method was used, it was not referred to. Further, selective data has been reported which makes it difficult to analyse the results independently and retrospectively review the trend over time. These shortcomings are compounded by the use of derived data (such as %FPF) to interpret the results which has a considerable biasing tendency in addition to masking the actual performance of the delivery system. Further, statistical analysis has not been reported in many studies while comparison of stage- or stage-groups has altogether been missing. Hence, comprehensive data analyses for *in-vitro* equivalence in the light of current regulatory requirements have been presented in this thesis.

Flow duration of 30 seconds at 28.3 L/min has been used in most studies. This is equivalent to 14.15 L of air passing through ACI. However, in this project, airflow for 8.5 seconds has been used to allow passage of 4 L through ACI so as to reflect on the inhalation volume of a healthy human adult (Hall et al., 2011).

In most reported studies, ACI impaction plates were not coated to prevent particle bounce and re-entrainment. However, this shortcoming has been addressed to in this project.

The recommended technique for inhalation from an MDI alone or with spacer requires press and breathe in the discharged puff simultaneously. Since *in-vivo* studies have been based on this inhalation manoeuvre; therefore, no inhalation delay methodology has been applied for *in-vitro* studies. Pursuant to this approach, tidal breathing and simulation profiles through spacer has not been considered in this project.

Different methods have been used to pre-treat spacers with divergent *in-vitro* findings, to which many outcomes of *in-vivo* studies are at variance. Further, the divergence in spacer pre-treatment method is also noted in PILs for the same spacer in different regions and between countries. Interestingly, FDA advises to wash spacer with water after detergent treatment. Because of conflicting evidence and the lack of consensus on pre-treatment method, the spacers used in this project have been treated with aqueous detergent solution followed by water rinsing and drip-drying.

2.8 Statements of Purpose

In-vitro dose delivery characteristics of Ventolin Evohaler®, Airomir® and Salamol® HFA will be investigated for equivalence in the light of the current regulatory requirements using clinically relevant dose of 2 puffs. Spacer studies will also be conducted using Volumatic, AeroChamber Plus and Able spacer. The round actuator mouth piece of Airomir does not fit into Volumatic, therefore, these studies will not be undertaken.

3 Chapter 3: Methodology

3.1 Overview

In Europe, regulatory authorities apply a step-wise approach for establishing equivalence between MDIs; starting from *in-vitro* comparisons, followed by pharmacokinetic (PK) studies and then pharmacodynamics (PD) studies (EMA, 2009; Dissanayake, 2010; Evans et al., 2012; Forbes et al., 2015; Lee et al., 2015). If equivalence is established at *in-vitro* level, no further studies are required; otherwise PK studies are to be conducted. If PK studies could not prove similarity between comparator MDIs then PD studies are to be carried out. Nevertheless, regulatory authorities may require further studies beyond *in-vitro* comparisons as they may deem necessary on reasonable grounds. On the other hand, FDA requires all of these studies to be performed and the comparator MDIs are considered equivalent only on the weight of evidence basis.

The current project will explore *in-vitro* and *in-vivo* equivalence of salbutamol MDIs. This chapter outlines the methods, techniques and procedures to be used to carry out the research work. Analytical, *in-vitro*, *in-vivo* and statistical methodology have been outlined separately.

3.2 Analytical HPLC Methodology

The development and validation will be carried out in compliance to ICH guidelines Q2(R1) (2005). Details of HPLC methods for aqueous and urine samples are provided in Chapter 4.

3.3 *In-Vitro* Methodology

Aerodynamic particle size distribution (APSD) profile of an MDI provides information on its dose delivery characteristics which may predict the likelihood and whereabouts of aerosol particle deposition in the respiratory tract. APSD profiles are generated using cascade impactors (BP, 2005; USP28-NF23, 2005; Ph. Eur., 2011; Mitchell and Nagel, 2003 & 2004; Christopher et al., 2007). An eight-stage Andersen Cascade Impactor (ACI) is widely used for the *in-vitro* determination of the APSD.

3.3.1 Materials and Methods

3.3.1.1 Materials and Equipments

Andersen Cascade Impactor	Mark II, Copley Scientific Ltd., UK.
Critical Flow Controller	Model TPK, Copley Scientific Ltd., UK.
GAST 1023 Pump	Brook Crompton, UK.
Air Flow Meter	Model PR 4000, MKS Instruments, MA, USA.
GF 50 filter	Copley Scientific Ltd., UK.
Silicone spray	Releasil B silicone spray, Dow Corning Ltd., U.K.
Parafilm M Laboratory film	Pechiney Plastic Packaging, USA.

3.3.1.2 MDIs

Ventolin Evohaler™, GlaxoSmithKline, UK
Airomir™, Teva, UK
Salamol™, Teva, UK

These MDIs deliver 100 µg ex-valve (metered dose) and 90 µg ex-actuator (emitted dose) per puff as salbutamol base equivalent. Their nominal strengths as salbutamol sulphate are 120 µg and 108 µg, respectively.

3.3.1.3 Spacers

Volumatic Spacer™, Allen & Hanburys Ltd., UK.
AeroChmaber Plus™, Trudell Medical International Europe Ltd., UK.
Able Spacer™, Clement Clarke International Ltd., UK.

The spacer images are shown in Figure 3.3.1 (not to scale).

(a) Volumatic Spacer (b) AeroChamber Plus (c) Able Spacer

Figure 3.3.1. Images of spacers.

3.3.2 *In-Vitro* Study Design

A designated ACI will be used to minimise variability from different lots (Stein and Olson, 1997; Stein, 1999). ACI experiments will be conducted with MDI alone and with a spacer, both randomly selected. Studies with Airomir will be performed with AeroChamber Plus only since its round mouthpiece does not fit in the oblong inhaler port of Volumatic. All MDIs will be stored on their sides while being used to reflect normal storage. All experiments to be performed under ambient environmental conditions. However, temperature and humidity in the laboratory is maintained between 18-23 $^{\circ}$C and 40-60 %RH, respectively. All MDIs, spacers, equipments and solvents are to be acclimatised to ambient conditions for 1 hour before use. Each experiment will be performed five times using two separate actuations on each occasion.

3.3.2.1 ACI Procedure

Pharmacopoeial methods (BP, USP, Ph. Eur.) are to be applied to determine the *in-vitro* particle size distribution of MDI alone and when used with a spacer. Clinically relevant dose of 2 puffs of salbutamol MDIs are to be used in this study. Protocol 3.3.1 describes procedures for preparing an MDI and spacer before testing and details of ACI testing are provided in Protocol 3.3.2. A summary of the testing procedures follows underneath.

Pre-Test Preparation:

Salbutamol MDIs are to be primed by discharging puffs to waste; 5 shots from new un-used MDI and 4 shots from in-use MDI. This is to minimise the probability of firing low content puff (Ross and Gabrio, 1999) and to comply with instructions in the patient information leaflet (PIL).

Canister valve and actuator are to be cleaned with deionised water and dried with methanol followed by air (Sheth et al., 2015).

Before each test, the spacer is to be washed in lukewarm mild detergent solution, rinsed with deionised water and left to drain and air dry. This pre-treatment minimises the electrostatic charge on the spacer (Table 2.4.2).

Impaction plates are to be coated evenly with silicone oil spray to minimise particle bounce and re-entrainment effect (Nasr and Allgire, 1995; Nasr et al., 1997; Mitchell, 2003; Mitchell and Nagel 2003; Guo et al., 2008; Sheth et al., 2015). The plates are to

be left for 15 minutes and then assembled. The assembled plates are to be used for testing in 30 min post-spray coating.

Test Procedure:

Previously cleaned, washed and dried ACI is to be assembled with silicone oil spray coated metal plates using designated stages (Protocol 3.3.1). The stages are to be stacked in reverse order of stage number, starting from placing back-up filter in the filter stage at the bottom (

Figure 3.3.2). A steady airflow through ACI is to be maintained at 28.3 ± 0.5 L/min (in-house limits) at the Induction Port (IP) using flow meter. Flow duration is to be set at 8.5 seconds on critical flow controller to allow 4 L of air to pass through. Primed MDI, shaken immediately before the experiment, are to be inserted into the IP using a designated adapter. The puff is to be discharged while simultaneously starting flow through the ACI (Chambers and Ludzik, 2008). Second puff is to be discharged as before after shaking for 5 sec, keeping a gap of 30 sec between the two puffs. After completing the flow duration, ACI is to be dismounted. The drug contents are to be recovered from the MDI and ACI components, stages and filter with deionised water and made up to volumes as shown in Table 3.3.1.

This experiment is to be repeated with pre-treated spacer attached to IP. The MDI puff is to be discharged into spacer at its inhaler port (Protocol 3.3.2). Aqueous washings, including that from the spacer are to be collected as mentioned earlier.

3.3.2.2 Sample Analysis

The sample solutions are to be assayed for salbutamol contents on HPLC using a validated method (Chapter 4).

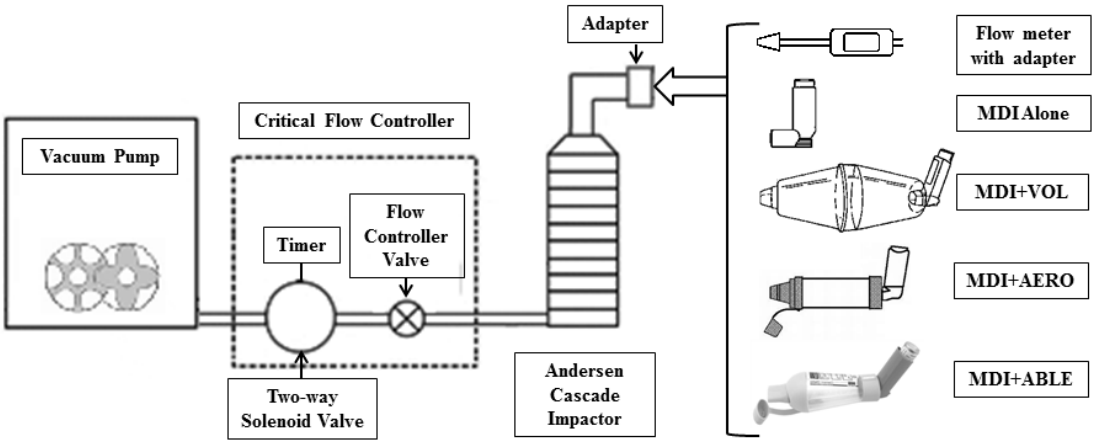


Figure 3.3.2. Schematic representation of ACI experimental set-up for Aerodynamic Particle Size Characterisation of an MDI without and with spacer.

Modified from USP (2005). VOL = Volumatic; AERO = AeroChamber Plus; ABLE = Able spacer

Table 3.3.1. Washing volumes used for various components and stages of ACI.

MDI and ACI Components / Stages	Volume (mL)
MDI Canister Valve (atomising nozzle)	50
MDI Actuator	50
Spacer & mouthpiece adapter	250
ACI Throat (no spacer used)	200
ACI Throat (spacer used)	50
ACI S-0	10
ACI S-1	10
ACI S-2	10
ACI S-3	50
ACI S-4	50
ACI S-5	50
ACI S-6	10
ACI S-7	10
ACI Filter	10

3.3.2.3 Protocol 3.3.1: Preparing an MDI, spacer and impaction plates for ACI testing

I. Priming of an MDI

Prime the new un-used MDI with 5 and in-use MDI with 4 shots to waste as follows:

1. Shake MDI for 5 seconds.
2. Uphold the MDI vertically and discharge 1st shot inside a card box (with paper towel covered inner walls) placed in the fume hood.
3. Repeat for 2nd and remaining shots as above allowing 30 seconds between shots.
4. Clean canister valve and actuator with de-ionised water and dry with methanol followed by air.

II. Preparation of spacers for testing

Before each test, wash the spacer in lukewarm mild detergent (1mL of detergent in 2L of deionised water), rinse with deionised water and leave to drain and air dry.

III. Preparation of ACI for Testing

1. Washing of Impaction Plates: Before (or after) each test, rinse all impaction plates with hot water. Then soak them in warm 1% detergent solution in water (e.g., 10:1000 mL) for 15 minutes. Rinse thoroughly with hot water, followed by deionised water and methanol and leave to dry.
2. Coating of Impaction Plates:
    a. Using tweezers, place previously washed and dried ACI stage plates on the designated shelved stand which holds plates vertically. Place this stand in fume hood.
    b. Coat plates uniformly by using silicon oil spray.
    c. After coating, lay the shelved stand horizontally outside the fume hood and leave for 15 minutes.
    d. Using tweezers, assemble coated plates into ACI stack.
    e. Place the entrance cone on top of assembled stages and hold these together with three spring clamps.
    f. Attach the Induction Port to the entrance cone.

3.3.2.4 Protocol 3.3.2: ACI Testing Procedure

I. ACI Experiment-MDI alone

1. Switch on pump, attach flow meter at Induction Port (IP) using adapter and adjust flow rate to 28.3 ± 0.5 L/min (in-house limits). Use parafilm if required. Make sure all tubing connections and adapter junctions are airtight and airflow is consistent.
2. Set time on Critical Flow Controller to 8.5 seconds to allow for 4 liters of air to pass through the ACI.
3. Shake the MDI for 5 seconds.
4. Attach the MDI to the IP using designated mouth piece adapter.
5. Fire 1st shot into ACI and start timer on Critical Flow Controller at the same time.
6. Remove MDI after 8.5 seconds of the shot.
7. Shake MDI for 5 seconds.
8. Attach MDI again to Induction Port via the adapter. Allow 30 seconds between 1st and 2nd shots.
9. Fire 2nd shot into ACI and start timer on Critical Flow Controller simultaneously.
10. Switch off pump after 8.5 seconds of the last shot.
11. Recover the dose deposited on MDI components and ACI assembly using de-ionised water as described below. Collect each washing into separate volumetric flasks and make up the volume as shown in Table 3.3.1.
    a) Remove MDI from IP. Take out MDI canister from the actuator. Wash them separately.
    b) Release three spring clamps from ACI stages and remove IP assembly (entrance cone, IP and MDI mouthpiece adapter). Wash each component. When used, collect spacer washings separately (See Section 3.3.2.4, Method II).
    c) Using stainless steel tweezers, transfer each impaction plate and the final stage filter into designated glass petri-dishes, add about 9 mL of de-ionised water and immerse completely. Place designated glass cover on each petri-dish and gently shake on a flat-bed shaker for 5 min. Wash impaction plates of stages S3, S4 and S5 with two additional lots of de-ionised water (~10 ml each).

12. After completing a single test run, wash ACI equipment and all its components with mild hot water paying special attention to stage jet holes. Rinse with de-ionised water followed by methanol and leave to air dry. Rinse impaction plates as described in Protocol 3.3.1 (see Section 3.3.2.3: III (1)).

II. ACI Experiment-MDI with attached spacer

Use Method I (Section 3.3.2.4) with the following additional adjustments for spacer:

1. Measure the air flow at Induction Port (IP) and adjust flow rate to 28.3 ± 0.5 L/min.
2. Attach spacer to IP with an adapter. If required, use parafilm to make connection of spacer to IP inlet airtight. Use an appropriate support such as a stand to keep spacer horizontal and flush with Induction Port inlet.
3. Follow the procedure as described in Method I from Nos. 2 to 12. In brief, after shaking MDI for 5 seconds, park it into the inhaler port of spacer. Fire the puff into spacer while simultaneously starting the Critical Flow Controller timer to allow airflow through ACI. Remove MDI from spacer after 8.5 seconds, shake it again for 5 seconds, and repeat as above. Recover dose deposited in MDI components and ACI assembly as described in Method I. Disband the spacer, wash each part and collect all rinsing into a volumetric flask (Table 3.3.1).

3.3.3 CQAs, Deposition Profiles and Data Analysis

EMA quality guideline (2006) enumerates *in-vitro* tests for MDIs to measure their Critical Quality Attributes (CQAs) (ICH Q8, 2009). EMA (2006 & 2009) also requires provision of full profiles of APSD and the amount of drug deposited on each stage or their justified groupings. The CQAs are described hereunder:

Total recovery: This is the sum of active drug amounts recovered from the canister valve (orifice), the MDI actuator, induction port (IP), spacer (when used), ACI stages and back-up filter. The amount recovered from IP includes the amounts recovered from the mouth piece adapter and the ACI entrance cone (Figure 2.3.1 and

Figure 3.3.2). This parameter provides information on the system suitability (Christopher et al., 2003) although not required by regulatory authorities.

Mass balance: This is the amount of active drug delivered from the canister valve, i.e., ex-valve, or the metered dose, and includes the amount recovered from the MDI

actuator, spacer (when used), IP, ACI stages and back-up filter. Mass balance serves as system suitability check (Christopher et al., 2003; EMA, 2006).

Total emitted dose (TED): This is the dose of active drug that is discharged ex-actuator (or ex-mouthpiece) i.e., the amount of active drug deposited in spacer (when used), IP, ACI stages and back-up filter.

Total delivered dose (TDD): For an MDI with attached spacer, this is the amount of active drug delivered ex-spacer and includes deposition on IP, ACI stages and back-up filter. For an MDI without spacer, TDD = TED.

Impactor mass (S0toF): This is the amount of active drug recovered from all ACI stages and back-up filter.

Fine particle dose (FPD): This is the amount of active drug deposited on stages 3 to 7 and back-up filter. FPD (or respirable dose) consists of particles of aerodynamic size < 5 µm in diameter. The effective cut-off diameter size of ACI stage 3 is 4.7 µm at the flow rate of 28.3 L/min (BP; USP; Ph. Eur.).

Fine particle fraction (FPF): This constitutes the fraction of the active drug having aerodynamic particle size <5.0 µm. FPF is calculated as the ratio of FPD to TED and/or TDD. Both parameters are also expressed as percentage.

Aerodynamic diameter (D) and Mass median aerodynamic diameter (MMAD): The D is the diameter of a sphere of unit density having the same terminal settling velocity as the particle at issue (EMA, 2009). MMAD corresponds to the diameter that divides the particle size distribution into two halves with respect to mass (Smyth, 2003; BP; USP; Ph. Eur.). MMAD can be derived by plotting the cumulative percentage of mass less than the stated cut-off aerodynamic diameters on probability scale versus aerodynamic cut-off diameter on log scale.

Geometric standard deviation (GSD): It is the ratio of median diameter to the diameter at ±1 SD from the median diameter (Laube et al., 2011). GSD can also be calculated alongside MMAD by using the following equation (BP; USP; Ph. Eur.):

$$\mathrm{GSD} = \sqrt{\frac{D_{84.13\%}}{D_{15.87\%}}}$$

3.3.4 APSD and ACI Stage Grouping

EMA guidelines (2006 & 2009) also offer options for comparing APSD either as per impactor stage or as justified grouped stages. APSD profile obtained from ACI provides information on particle size of aerosolized drug. Individual stages are linked to specific particle size or a size-range and correlate to human respiratory tract (HRT) deposition sites (Pritchard, 2001). The amount of inhaled drug which deposits in HRT or in certain regions of HRT is related to clinical effects (Weda et al., 2004; Usmani et al., 2005). However, it is difficult to distinguish the role of individual stages for efficacy and safety, since underlying physiological and pharmaceutical phenomena (such as differences in breathing patterns, patient's age, gender, disease state, HRT morphology, inhalation technique, aerosol velocity in entering the airways and plume geometry) play a critical role in lung deposition (Lipworth and Clark, 1997; Labiris and Dolovich, 2003; Rubin and Fink, 2005; Venegas et al., 2013). On the other hand, stage groups include a broad range of particle sizes and relate lung deposition to regions (Pritchard, 2001). Therefore, inhaled particles from a broad range of sizes may be involved in clinical effects. Hence, it is more likely that a group of stages rather than a specific single stage represents clinical efficacy and safety. The three salbutamol HFA MDIs under study are polydisperse suspensions and generate aerosol clouds that contain a range of particle sizes. Therefore, comparison of grouped stages as opposed to individual stages is more predisposed to predict clinically relevant *in-vitro* differences between them.

Thus, ACI components and stages are to be pooled into groups to mimic the likely regional deposition in HRT (Table 3.3.2). Similar stage groups were reported by others (Heyder et al., 1986; Pritchard, 2001; Asmus et al., 2003; Heyder, 2004; Dunbar and Mitchell, 2005; Guo et al., 2008 & 2013; de Boer et al., 2015; Nagel and Suggett, 2017; Hillyer et al., 2018).

ACI has traditionally been used to predict deposition in HRT. Induction Port (IP) (along with spacer when used) represents oropharynx. Higher IP (or combined spacer and IP) deposition may predict higher oropharyngeal deposition which is largely swallowed and absorbed from gastrointestinal tract (GIT). This may lead to salbutamol related systemic side effects. Group 1 stages (CPM) represent upper respiratory tract, in particular the laryngeal region. Group 2 stages (FPM) represent trachea, bronchi and bronchioles, i.e., upper and central airways and deposition in this region is linked to bronchodilation.

Group 3 stages (EPM) represent alveolar region (peripheral airways). EPM reaching to alveoli is largely exhaled if breath is not held for few seconds. Absorbed EPM may contribute to systemic effects.

Table 3.3.2. ACI Stage Grouping.

ACI Components / Stage Groups	**ACI Components / Stages**	**Particle Size (µm)**	**Likely Respiratory Tract deposition sites**
Induction Port Mass (IPM)	SP (when used), IP, Entrance Cone, IP (& SP) Adapter(s)	≥ 10	Oropharyngeal region
Group 1: Coarse Particle Mass (CPM)	S0 + S1 + S2	< 10 to ≥ 4.7	Pharyngeolarynx region; trachea
Group 2: Fine Particle Mass (FPM)	S3 + S4 + S5	< 4.7 to ≥ 1.1*	Trachiobronchial region * < 2 µm Alveolar region
Group 3: Extrafine Particle Mass (EPM)	S6 + S7 + Filter	<1.1 to ≥ 0.43	Alveolar region

Further, FPD comparison of MDIs is also to be carried out to determine *in-vitro* equivalence. Since, TDD ex-spacer contains high proportion of FPD, this provides a common ground for performance comparison between MDI alone and with a spacer (EMA, 2006). Not surprisingly, published work on salbutamol HFA MDI performance provides only selective CQAs data while APSD assessment as either individual stages or group of stages is altogether lacking. Hence FPD would be a common criterion to compare this work to others'. Also in this thesis, these comparisons are centred on data generated from ACI and NGI techniques due to their similarity (Mitchell et al., 2003; Kamiya et al., 2004).

3.3.5 Statistical Analyses - *In-Vitro* Studies

For *in-vitro* equivalence determination, data are to be logarithmically transformed. Mean ratio and 90% Confidence Interval (CI) are to be generated which are then to be exponentially reverted to numerals. The MDIs are to be considered *in-vitro* equivalent if the mean ratio of a CQA (performance parameter) is between 0.85 and 1.18, i.e., within ±15% of the reference product (EMA, 2006 & 2009). Mean difference at 95% CI is to be calculated from non-transformed data (normal scale). ANOVA tests are to be performed using Bonferroni corrections for post-hoc pair-wise multiple comparisons; $p < 0.1$ and $p < 0.05$ are to represent significant differences for ratios and mean differences, respectively. Statistical analyses are to be performed using SPSS (SPSS Inc., USA).

CQAs and derived parameters are to be calculated from ACI components and stage data using MS Excel (Microsoft Corporation). MMAD and GSD are to be determined using Copley Inhaler Testing Data Analysis Software (CITDAS, Copley Scientific, UK).

3.4 *In-Vivo* Methodology

Pulmonary deposition and systemic bioavailability of an inhaled drug can be characterised by pharmacokinetic (PK) studies (EMA, 2009). PK methods are indirect measurements from serum and urine (Chrystyn, 2001; Lu et al., 2015). These methods are useful in crossover studies to investigate the equivalence between inhaled products, inhalation treatment methods, devices and techniques.

It has been shown that 10-20 % of inhaled salbutamol from an MDI reaches the lungs while the remaining dose impacts on the oral cavity and oropharynx (Lipworth, 1996; Chrystyn, 2001). Since salbutamol is only negligibly absorbed from buccal mucosa (Lipworth et al., 1989a), most of it is eventually swallowed and subsequently absorbed through the GIT (Newman et al., 1981). The swallowed fraction undergoes pre-systemic metabolism in the intestine and first-pass degradation in liver (Ward et al., 2000). Only a small fraction (8%) of salbutamol is protein bound (Martin et al., 1971; Goldstein et al., 1987).

Following inhalation, salbutamol is delivered to the lungs within 1-3 seconds and is instantly absorbed (Shenfield et al., 1976). Since there is no first-pass conjugation in the lung (Shenfield et al., 1976; Ward et al., 2000), active (unchanged) salbutamol rapidly appears in the systematic circulation and is eliminated in the urine very quickly.

However, GI absorption of salbutamol is noticeably delayed as compared to lung absorption. It has been shown that following oral administration there were negligible amounts of salbutamol excreted in the urine within the first 30 min post-dosing (Hindle and Chrystyn, 1992; Silkstone et al., 2000). Further, Du et al. (2002) found two plasma peaks after inhaled salbutamol, 1^{st} at 15 min and the 2^{nd} at 2 hours when the mouth rinsing water was swallowed after inhalation. They attributed the 1^{st} peak to lung deposition and 2^{nd} peak to GI absorption of the swallowed mouth rinsing water. This is in line with the findings that 60% of systemic bioavailability of inhaled salbutamol was from the swallowed fraction (Ward et al., 2000) and that 50% of orally administered dose was absorbed from the gut reaching a peak at about 2 hours after intake (Goldstein et al., 1987).

It is clear that differences in the rate of absorption exist between the two absorption pathways. Hence, this lag time between quick lung absorption and delayed oral absorption of salbutamol has the potential to differentiate between lung deposition and systemic bioavailability (Hochhaus et al., 2015). Monitoring salbutamol in the urine over the first 30 min post-inhalation indicates how much salbutamol entered the systemic circulation through pulmonary absorption (Hindle and Chrystyn, 1992). Therefore, the first 30 min post-inhalation of salbutamol dose is considered as a sensitive index of the lung dose and indicates the relative bioavailability to the lungs (Hindle and Chrystyn, 1992). Pharmacokinetic studies using plasma differentiate between them by determining partial area-under-the-curve (AUCs) such as $AUC_{(0–0.5)}$, C_{max} and $t_{max(0-0.5)}$ (Singh et al., 2011; Moore et al., 2017). A number of investigators used first 20 min to represent this index (Lipworth and Clark, 1997, Anhøj et al., 1999, Mobley and Hochhaus, 2001; Du et al., 2002). However, $t_{max(0-0.5)}$ is measured at pre-set time points and therefore may not be a true t_{max} which may fall between those times (Weber et al., 2010; Evans et al., 2012).

Total systemic bioavailability of salbutamol is estimated by comparing plasma AUC data or urinary drug excretion (to infinity). Hindle and Chrystyn (1992) collected urine up to 24 h post-inhalation to assess total systemic delivery.

Salbutamol is a polar and basic molecule (The Merck Index, 2003) and therefore unaffected by changes in the urine pH (Chrystyn, 2001). Because of these physicochemical properties, its passive tubular reabsorption in the kidney is prevented which obviates the need to control urine pH.

Comparisons of relative lung and total systemic bioavailability give some indication of relative efficacy and safety, respectively, of different inhalation methods or products in crossover studies.

Urinary excretion of drug following the oral administration of charcoal identifies the total effective lung dose (Borgstrom and Nilsson, 1990). The charcoal block technique separates systemic delivery via the gastrointestinal and pulmonary routes by preventing GI absorption. The systemic exposure from this study serves as a surrogate for the local lung absorption and exposure. When GI absorption is prevented, the differences in the AUCs indicate differences in the dose reaching the airways. Further, EMA (2009) requires two PK studies for drugs with significant GI absorption such as salbutamol. One study is performed in the absence of charcoal to prove “safety equivalence” as the

total systemic exposure from both the lung and gut absorptions is measured. The second PK study is performed in the presence of activated charcoal to prove "efficacy equivalence" and only measures the "lung absorption exposure" as the absorption from the gut is inhibited by the charcoal. The charcoal blockade method therefore assesses the potential differences in pulmonary delivery. Hence, charcoal blockade studies have been often used in PK studies (Thorsson et al., 1994; Ward et al., 2000; Daley-Yates et al., 2001; Silkstone et al., 2000 & 2002b; Mazhar and Chrystyn, 2009; Abdelrahim et al., 2011; Singh et al., 2011; Said et al., 2012; Moore et al., 2017).

The application of plasma pharmacokinetic methods to inhalation studies is difficult. The small doses in large volume of distribution result in low systemic concentrations (Hindle and Chrystyn, 1992), which keep decreasing over time and present challenges for drug assay accuracy and reproducibility (Rogers and Ganderton, 1995). Also, sophisticated and costly analytical equipment may be required to quantify low plasma levels. Administering high doses may have implications for systemic effects (Lipworth et al., 1989; Fowler and Lipworth, 2001; Du et al., 2002) while not being clinically relevant. Hence, in these circumstances EMA (2010) would accept urinary excretion data as a surrogate for a plasma concentration-time profiles. In contrast, the concentrations of drugs in urine are much higher and can be assayed with simple analytical instruments using clinically relevant doses.

Further, plasma pharmacokinetic (PK) methods are invasive and require involvement and supervision of health care personnel which is not always feasible. Besides, these methods make it difficult to recruit healthy volunteers and patients. On the other hand, urinary PK methods are non-invasive and simple to carry out.

The PK method proposed by Hindle and Chrystyn (1992) uses urine concentrations to estimate the amount of inhaled drug. The amount of salbutamol excreted in the first 30 min identifies the relative lung bioavailability, and the cumulative amount of salbutamol and its metabolite excreted over the 24 hour post-inhalation identifies relative systemic bioavailability. This method has demonstrated linearity of dose-response relationship of inhaled salbutamol (Tomlinson et al., 1995; 2003) and inhaled terbutaline (Abdelrahim et al., 2011), and is reproducible (Hindle and Chrystyn, 1994; Tomlinson et al., 2003; Abdelrahim et al., 2011; Said et al., 2012). This method has been shown to be useful to compare inhaled salbutamol from MDI with oral salbutamol administration (Hindle and Chrystyn, 1992; Silkstone et al., 2000), spacers used with MDI (Chege and Chrystyn,

1994; Hindle and Chrystyn, 1994; Silkstone et al., 2002a; Mazhar and Chrystyn, 2008), DPIs (Hindle et al., 1995; Hindle et al., 1997; Chege et al., 1998) and nebulisers (Silkstone et al., 2000; Silkstone et al., 2002b; Mazhar et al., 2008). Moreover, this method can also differentiate between inhalation techniques (Hindle et al., 1993; Tomlinson et al., 2005) and formulations (Chege and Chrystyn, 2000). This method has also been useful to verify the correlation between lung deposition and improved spirometry (Chrystyn et al., 1998; Tomlinson et al., 1999 and 2005; Mazhar et al., 2008).

Further, this method has also been extended to other inhaled drugs, such as sodium cromoglycate (Aswania et al., 1999; Aswania and Chrystyn, 2001 & 2002), nedocromil (Aswania et al., 1998), gentamicin (Nasr and Chrystyn, 1997; Al-Amoud et al., 2005), formeterol (Nadarassan et al., 2007), terbutaline (Abdelrahim et al., 2011) and beclometasone (Said et al., 2012).

In the current project, this urinary PK methodology is being applied to salbutamol HFA MDIs and will be extended to spacers used with them.

3.4.1 Bioequivalence and regulatory requirements

There is ongoing debate on acceptable methodology and bioequivalence (BE) limits for PK studies of inhaled drugs (Parameswaran, 1999; Daley-Yates and Parkins, 2011; Fuglsang, 2012; Garcia-Arieta and Gordon, 2012; Evans et al., 2012; Hochhaus et al., 2015; Al-Numani et al., 2015; Lee et al., 2015; Lu et al., 2015). In addition, there are differences in the approach of regulatory bodies in Europe and other continents. EMA guideline (2009) states that PK studies be conducted in patients, while FDA recommends these studies are performed in healthy volunteers. Interestingly, EMA guideline (2010) suggests that the model of healthy volunteers is adequate in most instances to detect formulation differences and to extrapolate the results to other populations (e.g., the elderly, children). However, the rare instances where the extrapolation is not adequate are not identified. Therefore, in the present situation, it can be assumed that the model of healthy volunteers is applicable (García-Arieta and Gordon, 2012). It may be that those with asthma may have different airway deposition. Nevertheless, it has been shown that the only difference between healthy volunteers and those with asthma is that lung deposition is related to airway calibre (Lipworth and Clark, 1997) and therefore bioequivalence studies are often conducted in healthy

subjects (Daley-Yates and Parkins, 2011). Furthermore, Hochhaus et al. (2015) report that EMA is currently accepting these studies in healthy volunteers.

Healthy volunteer PK studies are likely to be at least as, or more, sensitive than patient studies to detect differences between different treatment methods, inhalation techniques and formulations (Dissanayake, 2010). Also, the model of healthy volunteers avoids the influence of variable airways obstruction across study periods in asthma and COPD patients. Besides, the urinary salbutamol pharmacokinetic method has been shown to be more sensitive to detect a difference in relative lung deposition than the methacholine challenge method recommended by Regulatory Authorities (Tomlinson et al., 2003). Hence, it is reasonable to preferentially undertake PK studies in healthy volunteers. Therefore, healthy volunteers will participate in the current study. Further, EMA recommends the conduct of the BE study with and without the use of spacers.

3.4.2 Bioequivalence criterion

Regulatory limits for the bioequivalence of inhaled products are that the 90% confidence limits should be between 0.80–1.25 for C_{max} and AUC (EMA, 2009 & 2010). However, it has been suggested that when comparing relative potencies these limits should be between 0.67 and 1.50 (EMA, 2009; Parameswaran, 1999). Hence, the urinary PK data in this project will be assessed in line with both these limits.

Much larger numbers of subjects may need to be studied to make firmer conclusions to suggest comparability between inhaled products. However, most of the studies that were included in a meta-analysis comparing different inhalation methods used a low number of subjects (Brocklebank et al., 2001), and were designed to show equivalence. Hence, in this project, a minimum number of 12 evaluable subjects will be included which is in compliance to EMA (2010) recommendations for any BE study.

3.4.3 Subject selection

EMA requires that subjects should be ≥18 years of age and preferably have a Body Mass Index (BMI) between 18.5 and 30 kg/m^2, and that they could belong to either sex.

Healthy and non-smoking volunteers from either sex will be selected for the PK study. Smokers will be excluded as they may introduce variability due to having an altered mucociliary clearance and local microenvironment (Scott, 2004) and induced

metabolism (Kroon, 2007; Olsson et al., 2011). Besides, pregnant women will be excluded.

This study is to be conducted in accordance with Good Clinical Practice and the guiding principles of the Declaration of Helsinki (World Medical Association (WMA), 2013).

3.4.4 Subject training

Training of the subjects for correct and consistent inhalation technique is essential to minimise variability in the dose delivery that is not related to the treatment method. All healthy volunteers will therefore be trained to use the following inhalation technique (Hindle et al., 1993; Laube et al., 2011; PILs):

3.4.4.1 MDI alone inhalation technique

1. Take the cap off.
2. Shake the primed MDI for 5 seconds (see Protocol 3.3.1 (3.3.2.3) for priming).
3. Exhale slowly, as far as comfortable (to empty the lungs).
4. Hold the inhaler in an upright position.
5. Immediately place the inhaler in the mouth between the teeth, with the tongue flat under the mouthpiece.
6. Ensure that the lips have formed a good seal with the mouthpiece.
7. Start to inhale slowly, through the mouth and at the same time press the canister to actuate a puff.
8. Maintain a slow and deep inhalation for 5-10 seconds, through the mouth, until the lungs are full of air.
9. At the end of the inhalation, take the inhaler out of the mouth and close the lips.
10. Continue to hold the breath for as long as possible, or up to 10 seconds before breathing out slowly.
11. Breathe normally.
12. Repeat steps 2–10 for the second puff after 30 seconds.

3.4.4.2 MDI with the attached Spacer

1. Take the cap off.
2. Shake the primed MDI for 5 seconds (see Protocol 3.3.1 (3.3.2.3) for priming).
3. Insert the mouthpiece of the MDI into the open end of the spacer (MDI port) and ensure a tight fit.

4. Exhale slowly, as far as comfortable (to empty the lungs).
5. Place the mouthpiece of the spacer in the mouth with the teeth over the mouthpiece and the lips sealed around it.
6. Actuate one puff into the spacer and simultaneously start to inhale slowly through the mouthpiece.
7. Maintain a slow and deep inhalation for 5-10 seconds through the mouth, until the lungs are full of air.
8. At the end of the inhalation, take the spacer out of the mouth and close the lips.
9. Continue to hold the breath for as long as possible for up to 10 seconds before breathing out slowly.
10. Breathe normally.
11. Repeat steps 2–9 for the second puff after 30 seconds.

3.4.5 *In-Vivo* Study Design

This bioequivalence single dose cross-over open study has been approved by Bradford University Ethics Committee. Initially, 14 non-smoking healthy volunteers (7 females) older than 18 years with an average FEV1 > 90% of predicted participated after giving informed written consent. However, one male volunteer discontinued due to illness which was not study-related.

All volunteers are to be trained in optimal inhalation technique (Sections 3.4.4.1 & 3.4.4.2) and are to be refreshed on the standard technique on the day of study before inhaling the dose.

All salbutamol MDIs are to be primed immediately before each study and detergent pre-treated spacers are to be used (Protocol 3.3.1 (3.3.2.3)).

The PK study is to consist of two parts. In Part 1, each volunteer is to inhale 2 puffs from the primed salbutamol MDI in two separate manoeuvres. In Part 2, each volunteer is to repeat this study with the concurrent administration of activated charcoal by swallowing 100mL of charcoal slurry immediately before and after two inhalations (Activated Charcoal 25g in 200mL of water; Carbomix^{TM}, Penn Pharmaceuticals, UK) (Silkstone et al., 2000; Mazhar and Chrystyn, 2009). Volunteers are to swish around charcoal slurry in the mouth before swallowing all of it. Each study is to be separated by 7 days to allow for wash-out of salbutamol.

Urine samples are to be collected 0.5 hour before and after inhalation, the post-dose sampling time to start after inhaling the 1st puff. Thereon total urine is to be pooled for 24 hours. Volume and pH of all urine samples is to be recorded. All urine samples are to be stored at –20°C till extracted and assayed.

The residual dose in MDI actuator and spacer (when used) is to be assayed after inhaling two puffs (Protocol 3.3.2 (3.3.2.4)).

3.4.5.1 Bioequivalence of Salbutamol HFA MDI alone

This is a six way study in two parts. Each part of this study consists of three sub-sets involving three different salbutamol MDIs, vis-à-vis: Ventolin Evohaler®, Airomir® and Salamol®. Each volunteer is to inhale 2 puffs in two separate manoeuvres (as detailed in Section 3.4.4.1) from one of the randomly selected primed MDI.

3.4.5.2 Bioequivalence of Ventolin Evohaler without and with spacer

This is two parts eight way study. Each part of this study consists of four sub-sets involving four treatment methods of salbutamol inhalation from either Ventolin Evohaler alone (Evo) or attached to one of the three pre-treated spacers: Volumatic® (VOL), AeroChamber Plus® (AERO) or Able® (ABLE).

Each volunteer is to inhale 2 puffs in two separate manoeuvres (as described in Section 3.4.4.2). Each single dose discharged into a spacer is to be inhaled within the first second of discharge into one of the randomly selected spacer.

3.4.5.3 Bioequivalence of Airomir Without and with AeroChamber Plus

This is a four way two parts study. Each part of this study consists of two sub-sets involving two methods of salbutamol inhalation, vis-à-vis: Airomir alone (Airo) and with AeroChamber Plus (AERO). Volunteers to inhale 2 puffs as narrated in Section 3.4.4.2.

3.4.5.4 Bioequivalence of Salamol without and with spacer

This is a six way study split in two parts. Each part of this study consists of three sub-sets involving three treatment methods of salbutamol inhalation from either Salamol (Sala) alone or attached to VOL and AERO. Every volunteer to inhale 2 puffs as explained in Section 3.4.4.2.

3.4.6 Sample Analysis

Urine samples are to be processed and assayed for salbutamol using validated SPE and HPLC methods described in Chapter 4.

3.4.7 Statistical Analysis – *In-Vivo* Studies

Statistical comparisons are to be made between all treatment methods; i.e., MDIs, MDI alone and MDI with spacer, and between spacers. For *in-vivo* equivalence determination, data are to be logarithmically transformed. Mean ratio and 90% Confidence Interval (CI) are to be generated which are then to be exponentially reverted to numerals. Two tier assessment of bioequivalence of the treatment methods is to be carried out, one at ±20% (0.80 – 1.25) and the other at ±33% (0.67 – 1.50). The comparative treatment methods are to be considered *in-vivo* equivalent if the mean ratio of parameters under study (USAL0.5 and USAL24) is within one of these limits (EMA, 2009). Mean difference at 95% CI is to be calculated from non-transformed data (normal scale). ANOVA tests are to be performed using inhalation method as fixed and volunteers as random factors. Bonferroni corrections are to be applied for post-hoc pair-wise multiple comparisons; $p < 0.1$ for mean ratios and $p < 0.05$ for mean differences are to represent significance.

A paired t-test with 95% CI is to be used to compare recoveries of inhaled salbutamol without and with charcoal blockade and $p < 0.05$ is to be considered a significant difference.

The delivered dose to the volunteers is to be estimated by subtracting the dose that is retained in the MDI components and spacer (UDD) from the nominal dose (ND) (200 µg) [ND – UDD].

Statistical analyses are to be performed using SPSS v22 (SPSS Inc., Chicago, USA).

3.4.8 Summary of Methodology

The research in this project will have three components vis-à-vis: analytical method development for inhaled salbutamol, *in-vitro* characterisation of salbutamol MDIs without and with spacer, and their *in-vivo* equivalence. Analytical method development will be carried out as per ICH guidelines (Q2R1) while *in-vitro* and *in-vivo* equivalence will be assessed in line with EMA guidelines (2006, 2009 & 2010).

4 Chapter 4: Development and validation of salbutamol HPLC assay

4.1 Overview

Salbutamol is a widely prescribed β_2-agonist for relieving bronchospasm in patients with asthma and Chronic Obstructive Pulmonary Disease (NICE, 2010 & 2017; Moxham and Costello, 1997; Pride, 1987). Salbutamol is first-pass metabolised to an inactive sulphate conjugate in the liver and possibly in the gut wall (Morgan et al., 1986). It is rapidly absorbed from the gastro-intestinal tract and excreted in the urine as an unchanged drug or metabolites. On inhalation, about 10 to 30 % of the salbutamol dose is absorbed from the lung and most of the remaining inhaled dose is swallowed and absorbed from the gut (Chrystyn, 2001). It is suggested that salbutamol does not metabolise in the lung (Hindle and Chrystyn, 1992). The amounts of salbutamol recovered as unchanged or metabolite fraction determines the proportion of inhaled salbutamol relative to the proportion swallowed. Since only unchanged salbutamol is effective in relieving the acute attacks of bronchospasm rapidly, it is therefore rational to quantify the amounts of both unchanged and metabolised salbutamol so as to identify its relative lung deposition. Relative lung deposition is used as a yardstick in inhalation bioequivalence studies.

A urinary salbutamol pharmacokinetic method has been identified to compare the relative lung and systemic bioavailability of different inhaled products and inhalation techniques (Hindle and Chrystyn, 1992). Although several reversed phase high performance liquid chromatography (HPLC) methods have been reported to determine salbutamol in biological fluids for studying its pharmacokinetics in healthy volunteers and patients (Rejeev et al., 1982; Hutchings et al., 1983; Morgan et al., 1986; Miller and Greenblatt, 1986; Ong et al., 1989; Lipworth et al., 1989; Hindle and Chrystyn, 1992; He and Stewart, 1992; Gupta et al., 1994; Clark et al., 1996; Mohamed et al., 1999; Murthy and Hiremath, 2004), only three have used a urine matrix (Morgan et al., 1986; Clark et al., 1996; Hindle and Chrystyn, 1992). Since urine matrix has more endogenous interfering peaks as compared to plasma/serum, it was not possible to use assay methods developed for plasma/serum matrix. In addition, collection of blood from healthy volunteers and patients require invasive techniques which limits their participation in the clinical trials. Morgan et al. (1986) worked at low sensitivity because of the presence of interfering peaks in urine while Clark et al. (1996) did not hydrolyse urine samples to assay the salbutamol ester metabolite. Both methods do not use an internal standard.

The analysis method of Hindle and Chrystyn (1992) has been widely used to identify the relative lung and systemic bioavailability following an inhalation (Chrystyn, 2001). However, the run time of this assay is long (>50 min) because of the need to split some interferences with the salbutamol and the internal standard (bamethane) peaks which frequently could not be baseline resolved. Also, the SPE method produced variable recoveries of salbutamol and bamethane. Further, these recoveries were very low (≤30% and ≤40%, respectively) from the hydrolysed urine samples. The aim of this study was therefore to overcome these difficulties. Hence a new HPLC method with a reasonably short run time and two new solid phase extraction (SPE) methods have been developed, optimised and validated using terbutaline as the internal standard. The first SPE method uses mixed-mode cationic cartridges but can only be used for unchanged salbutamol. The second method uses polymeric cartridges and can be used for both unchanged and total salbutamol (unchanged plus metabolised). Since many studies only use data for unchanged salbutamol in the first 30 min then the first method is recommended because the extraction cartridges are much cheaper and the sample preparation time is shorter. The application of the assay to quantify salbutamol excreted in urine from participants of an inhaler study is reported.

4.2 Experimental

4.2.1 Materials and Reagents

All solvents used for chromatography and SPE were of HPLC grade (BDH, UK). Reagent grade orthophosphoric acid (H_3PO_4, 85%, specific gravity 0.85), 7 N hydrochloric acid (HCl), ammonia solution (35%, specific gravity 0.88), sodium dodecyl sulphate (Biochemical; SDS), potassium dihydrogen phosphate (KH_2PO_4) and potassium hydroxide pellets (KOH) were also obtained from BDH (UK). Ultra-purified (Deionised) water was prepared in-house using a Milli-Q Reagent Water System (Millipore). Salbutamol base, terbutaline hemisulphate, bamethane and other drugs and compounds tested for method specificity and selectivity were purchased from Sigma-Aldrich (UK).

4.2.2 Preparation of Reagents for SPE

Stock solution of KH_2PO_4 (0.5 M, pH 7.0) was prepared, pH adjusted with KOH (10 M) and filtered (0.45 μm). Working buffers of 100, 60, 45, 30 and 15 mM were prepared from the stock buffer solution. Another aliquot of 0.5 M KH_2PO_4, pH 13.0 was

prepared for use in post-hydrolysis SPE. All buffers were stored at ambient temperature (25-27°C) and used within 3 months.

Stock solution of HCl (1 N, ±0.2%) was prepared by diluting HCl (~7 N, BDH Convol) in water to make 1 L. Working HCl strengths (0.00001 N, pH ~5.0; 0.005 N, pH ~2.50; 0.01 N, pH ~ 1) were made from diluting 1 N HCl in purified water. All dilutions were used within 6 months and the stock solution (1 N) in one year.

Aqueous solutions (v/v) of methanol (MeOH, 5%), acetonitrile (ACN, 2%), tetrahydrofuran (THF, 0.25%) and acetic acid (CH_3COOH, 2%) were prepared fresh every week. Ammoniacal methanol (6% v/v, pH ~12.0) was prepared fresh on the day by adding ammonia solution (6 mL) to HPLC grade methanol (25 mL).

4.2.3 Preparation of Urine and Aqueous Standards

Blank urine was obtained from 14 (7 females) volunteers to prepare stock and working standard solutions. Stock aqueous solutions of 10,000 μg/L for salbutamol base and 5000 μg/L for terbutaline sulphate were prepared. Stock salbutamol urine standard (1000 μg/L) was prepared by diluting appropriate volume of the aqueous stock solution with urine. From this urine stock solution working urine standard solutions containing salbutamol concentrations of 750, 500, 300, 250, 200, 150, 100, 75, 50, 25, 15, 10 and 5 μg/L were prepared by serially diluting urine standards with urine. Quality Control (QC) standards (200, 100 & 50 μg/L) were prepared from a separate stock solution. All urine standard solutions and blanks were transferred to polypropylene tubes (25 mL) and frozen at −20°C. Previous studies have shown that when prepared and frozen at −20°C, these standards were stable for 12 months (Tomlinson, 2000). All standard solutions were therefore frozen and used within 12 months. Terbutaline sulphate aqueous solution (500 μg/L) was used as the internal standard *in situ* during the SPE. Salbutamol aqueous standards were prepared in parallel concentrations each containing 500 μg/L terbutaline sulphate. All aqueous standards were stored at 5°C.

4.2.4 Solid-phase extraction methods

Varian Vac Elute workstations (10 cartridge ports) with on-line laboratory vacuum were used for extraction. Eluates were dried under nitrogen (N_2) in the fume hood using a

Techne Dri-Block DB-3A sample concentrator, reconstituted with 1 mL mobile phase, thoroughly mixed and transferred to autosampler vials (screw-capped with air-tight seals) for injection onto the HPLC system.

4.2.4.1 SPE Pre-Hydrolysis (USAL METHOD)

Solid phase extraction (SPE) of unchanged salbutamol was carried out using mixed-mode cationic-exchange Isolute Confirm HCX 130 mg cartridges with a 10 mL capacity (IST, UK). The extraction procedure is described in Table 4.2.1. To each urine sample (1 mL), internal standard terbutaline (1 mL) was added. Similarly, in the blank urine sample, purified water was added instead of the internal standard. The samples were allowed to run through under gravity. Care was taken not to let the cartridges dry in any step before sample application.

4.2.4.2 SPE Post-Hydrolysis (USALMET METHOD)

Oasis HLB 30 mg in 1 mL (Waters, UK) polymeric cartridges were used for the extraction of total salbutamol (unchanged plus metabolised) and the internal standard. To each cartridge, a 25 mL reservoir (IST, UK) was attached at the top using an adapter (Supelco, UK). The flow rate of sample application and elution was maintained between 1-2 mL/min using vacuum while low vacuum was applied throughout the SPE.

The urine samples were first hydrolysed to convert all metabolised salbutamol back to the free salbutamol. The hydrolysis procedure consisted of boiling for 1 hour in a water bath a solution of 1 mL urine sample and 1 mL internal standard terbutaline in a glass test tube to which 8 mL of 0.1 N HCl was added and vortex mixed (10 mL). The test tubes were covered with kitchen foils and it was ensured that the meniscus of the test tube solution was well dipped into the boiling water. Blank urine was treated similarly except that 1 mL of purified water was added instead of the internal standard. After acid hydrolysis, the samples were left to cool and then 1 mL of 0.5 M KH_2PO_4 (pH: 13.0) was added and vortex mixed. The pH of the neutralised hydrolysate (11 mL) was checked randomly. The extraction procedure is described in Table 4.2.2.

Table 4.2.1. Sample pre-treatment and SPE method for salbutamol and terbutaline from un-hydrolysed urine (USAL METHOD).

SPE Pre-Hydrolysis (USAL METHOD) **Sample/Blank Pre-Treatment**	
1 mL Sample/Blank urine 1 mL Internal Standard/1 mL H_2O in Blank urine 2 mL 30 mM KH_2PO_4 (pH: 7.0) Mix and check final pH if necessary and randomly Total Volume: 4 mL	
Vol. (mL)	SPE PROCEDURE Solvents/Reagents
1.0	MeOH
1.0	15 mM KH_2PO_4 (pH: ~7.0)
4.0	Pre-treated Sample/Blank
2.0	15 mM KH_2PO_4 (pH: ~7.0)
2.0	HCl (0.00001 N, pH: ~5.0)
	Dry for ~ 2 min under full vacuum
1.0	HCl (0.005 N, pH: ~2.50), apply low vacuum
	Dry for ~ 5 min under full vacuum
1.50	MeOH:H_2O (75:25)
	Dry for ~ 5 min under full vacuum
	Insert glass test tubes for eluate collection
1.0	NH_3:MeOH (06:94), apply low vacuum for ~2-3 min when all eluted
	Concentrate at 60^0C under N_2 for ~15 min
1.0	Reconstitute with mobile phase, mix and transfer to autosampler vials, screw-capped with air-tight seals, for injection onto the HPLC system

Table 4.2.2. Sample pre-treatment and SPE method for salbutamol and terbutaline from hydrolysed urine (USALMET METHOD).

SPE Post-Hydrolysis (USALMET METHOD)

Sample/Blank Pre-Treatment

1 mL Sample/Blank Urine

1 mL Internal Standard/1 mL H_2O in Blank urine

8 mL 0.1N HCl, vortex mix, cover with kitchen foil, make sure sample meniscus is well dipped in boiling water, boil for 1 hour, cool

1 mL 0.5 M KH_2PO_4 (pH: 13.0), vortex mix

Check final pH if necessary and randomly: 6.5 - 7.2

Total Volume: 11 mL

Vol. (mL)	SPE PROCEDURE Solvents/Reagents
2.0	MeOH
2.0	45 mM KH_2PO_4 (pH: ~7.0)
11.0	Pre-treated Sample/Blank
2.0	15 mM KH_2PO_4 (pH: ~7.0)
	Dry for ~ 2 min under full vacuum
1.0	MeOH:H_2O (05:95
	Dry for ~ 1 min under full vacuum
1.0	ACN:H_2O (02:98)
	Dry for ~ 1 min under full vacuum
1.0	THF:H_2O (0.25:99.75)
	Dry for ~ 2 min under full vacuum
	Insert glass test tubes for eluate collection
2.0	CH_3COOH: H_2O (02:98), apply low vacuum and continue for ~2-3 min when all eluted
	Concentrate at 120^0C under N_2 for ~35 min
1.0	Reconstitute with mobile phase, mix and transfer to autosampler vials, screw-capped with air-tight seals, for injection onto the HPLC system

4.2.5 Optimised HPLC Method

The HPLC system and optimised HPLC method are detailed in Table 4.2.3.

Table 4.2.3. HPLC system and chromatographic conditions.

1. HPLC System	
Pump	Gilson 307
Degasser	Membrane Degasser, Thermal Separation Products
Column Thermostat	Column Chiller Model 7950, Jones Chromatography, UK.
Autosampler	SIL-9A Autosampler, Shimadzu, Japan.
Column	Zorbax, ODS 5 μm, 25 cm x 0.46 mm ID; Phenomenex, UK.
Guard Column	Security Guard cartridge, ODS 4 mm x 3 mm ID; Phenomenex, UK.
Detector	Spectroflourometric Detector RF-551 (Ver. 2.4, 12 μL flow cell); Shimadzu, Japan. Detector settings: Response time 1.5, Range x 128, Sensitivity High, Gain 1 and signal output at 1mV full scale
Integrator	Shimadzu Chromatopac CR-6A with attenuation set on 8 using Method 2061, Format 0.0 (zero) and signal input from the detector at 1 mV
2. Chromatographic conditions	
Mobile Phase (MP)	Acetonitrile:Tetrahydrofuran:Methanol:Buffer (B) with respective ratio of [10:08:14:68 (% v/v/v/v)]
Buffer (B)	KH_2PO_4, 5 mM, pH 2.50
Ion Pairing Agent	Sodium Dodecyl Sulphate 25 mM (Dissolved in B)
Filtration and Degassing	The mobile phase was filtered (0.45 μm, Millipore) and degassed by sonication (Decon FS200 B) under vacuum for 10 min.
Excitation: Emission	269:312 (nm)
Injection Volume	100 μL using 200 μL loop
Flow Rate	1 mL/min
Temperature	$30^{\circ}C$
Working Pressure	145-150 Bar

4.2.6 Validation

The efficiency of chromatographic separation and solid-phase extraction was evaluated and validated according to criteria described in the literature (Szepesi et al., 1989; Buick et al., 1990; Causey et al., 1990; Shah et al., 1992; CDER, 1994 & 2013; EMA, 2012; ICH-Q2(R1), 2005).

4.2.6.1 Validation of HPLC

Selectivity was determined by injecting:

a) blank mobile phase, Milli-Q water and blank urine collected from volunteers 0.5 hour before a salbutamol inhalation,
b) aqueous and urine standards, and
c) volunteers' urine samples collected 0.5 and 0.5-24 hour after salbutamol inhalation. Both un-hydrolysed and hydrolysed urine specimens were extracted and assayed.

Intra-day accuracy and repeatability (RSD of peak height ratio) were determined by injecting three salbutamol concentrations (50, 100 and 200 μg/L; QC Standards) in triplicate. The accuracy and precision of the calibration curves to measure the unknown concentrations or QC standards were determined by drawing calibration curves excluding these concentration points and expressed as mean measured concentrations (with their respective biases) and RSD.

The inter-day accuracy and precision were determined for all concentrations of the standard calibration curve. Inter-day accuracy (n=6) was determined as mean measured concentration of each calibration point, which was obtained using a linear regression equation of each calibration curve and reported as bias. Inter-day precision (n=6) was expressed as the RSD of the peak height ratios of individual calibration points.

Limits of Detection (LOD) and Quantitation (LOQ) were calculated using the data obtained from linear regression equations (n=6) (ICH-Q2(R1), 2005). LOD and LOQ calculated by linear regression were subsequently validated by the repeated analysis of three salbutamol aqueous and urine standards prepared at concentrations near the LOD (5, 10 and 15 μg/L) and the RSD was calculated (ICH-Q2(R1), 2005).

The ruggedness of the assay was estimated by day-to-day variability of the chromatographic response of three salbutamol urine QC standards and one unknown sample (volunteer KA24) evaluated in duplicate on 5 different days. Robustness was estimated by varying various HPLC conditions such as the mobile phase constituents, temperature, buffer strength and molarity of the ion-pairing agent.

4.2.6.2 Validation of extraction recovery, precision and accuracy

Intra-day recovery of salbutamol was determined by repeated SPE (n=3) of three urinary salbutamol QC standards selected at high, mid and low points of the calibration range (50, 100 and 200 μg/L). The inter-day extraction recovery (n=6) was determined using the extraction and assays of the whole calibration curve standards. The peak heights of salbutamol urine extracts were compared to the peak heights obtained with the direct injections of salbutamol aqueous standards assuming 100% recovery in order to provide an estimate of the extraction recovery. The intra- and inter-day accuracy and precision were determined as the percent relative recovery and RSD, respectively.

The study to optimise the conditions for the SPE when more than 1 mL of urine sample was used, consisted of extracting and injecting 8 replicates of hydrolysed salbutamol urine standards (50 μg/L) and a volunteer's 0.5-24 hour urine sample (NK24) for each test volume (1-5 mL) after the volunteer had inhaled 5 puffs of salbutamol from an MDI (Ventolin Evohaler™). The results were expressed as the recovered amount, bias and RSD.

4.2.6.3 Stability studies

Two stability studies were carried out, one for establishing the stability of salbutamol and terbutaline urine concentrates in the mobile phase post-reconstitution and post-SPE, and the other for concentrated urine extracts frozen at ─20°C for up to 40 days for later reconstitution.

The first study involved determining the stability of urine concentrates in the mobile phase over 0-38 hour after reconstitution at ambient temperature (25-27°C). This was assessed by repeated (n=5) HPLC determinations of three urine and aqueous salbutamol QC standards (50, 100 and 200 μg/L), a volunteer's urine sample containing no salbutamol (blank) and a 0.5-24 hour post salbutamol inhalation urine sample.

The second stability study was carried out to determine the recovery of extracted salbutamol and terbutaline in frozen urine concentrates for a period of 10-40 days post-SPE using the same QC standards and samples and the frequency of SPE as mentioned in the first study. The concentrated urine extracts collected as SPE eluates in glass test tubes were sealed with parafilm, further enveloped in polythene bags and frozen at ─20°C till defrosted and assayed. The first set of samples was considered as reference day-1 with no freezing.

The urine salbutamol QC standards, volunteer's 0.5-24 hour post-inhalation samples and blanks were all extracted the same day in duplicate using the SPE USAL METHOD. Accuracy (recovery) and precision were determined against aqueous standards to establish the stability at each test point.

4.2.7 Volunteer Study

This study was conducted as enumerated in Section 3.4.5 (Chapter 3 Methodology). In brief, fourteen healthy volunteers (7 females) participated in two parts of the study. In Part 1, on separate study days (one week apart), each volunteer inhaled 2 puffs (200 μg) from one of the randomly selected salbutamol MDIs, vis-à-vis: Ventolin Evohaler™, Airomir™, Salamol™ and AirSalb™ (Sandoz Ltd., UK). The MDI was randomly selected. In Part 2, each volunteer repeated this study with charcoal intake immediately before and after the two inhalations. All volunteers inhaled salbutamol from all MDIs.

On all occasions urine samples were collected 0.5 hour before and after inhalation and thereafter pooled their urine for 24 hours. The volume and pH of all urine samples were recorded. Aliquots of each urine samples were stored at –20°C till extracted and assayed. Urine samples collected at 0.0-0.5 hour post inhalation were assayed for unchanged salbutamol (USAL0.5) using the USAL METHOD. Pooled urine samples collected during 0.5-24h were assayed for their unchanged salbutamol (USAL24) using the USAL METHOD and for their salbutamol plus metabolite concentration (USALMET24) using the USALMET METHOD.

4.3 Results and Discussion

4.3.1 Validation of HPLC method

4.3.1.1 Wavelength Scanning

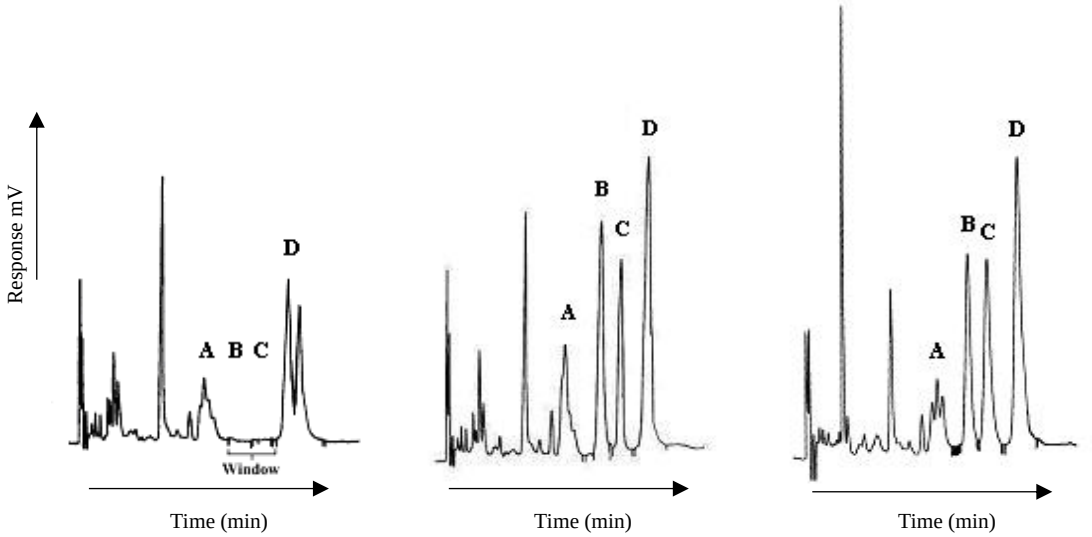
A decrease in sensitivity and eventual loss of response was noted at excitation:emission wavelengths (Ex:Em) 276:609 nm (Hindle and Chrystyn, 1992) with different combinations of solvents for mobile phase. To accommodate solvent effects, wavelength scans for salbutamol using HP 1100 Series with HP ChemStation for LC (Rev. A.06.01 [403]) revealed various Ex:Em combinations vis-à-vis: 220:312; 270:312; 220:614 and 270:614. These Ex:Em wavelengths are comparable to those found by other researchers (Gupta et al., 1994; Koh, 2003). At 220:312 baseline was noisy while at 220:614 and 270:614 the response decreased by 30% and 40% respectively when compared to 270:312. After multiple salbutamol injections around 270:312, the optimised Ex:Em combination of 269:312 was selected using Shimadzu Spectroflourometric Detector.

4.3.1.2 Representative Chromatograms

The representative HPLC chromatograms of un-hydrolysed and hydrolysed blank and a 0.5-24 hour volunteer's urine samples are shown in Figure 4.3.1 and Figure 4.3.2, respectively. Salbutamol eluted after ~24 min (RSD 0.31%; range 24.2-24.5 min) and terbutaline in ~27 min (RSD 0.26%; range 26.9-27.1 min) (n=50) with baseline resolution (R_s=1.8, RSD=3.3%, n=5).

4.3.1.3 Specificity and Selectivity

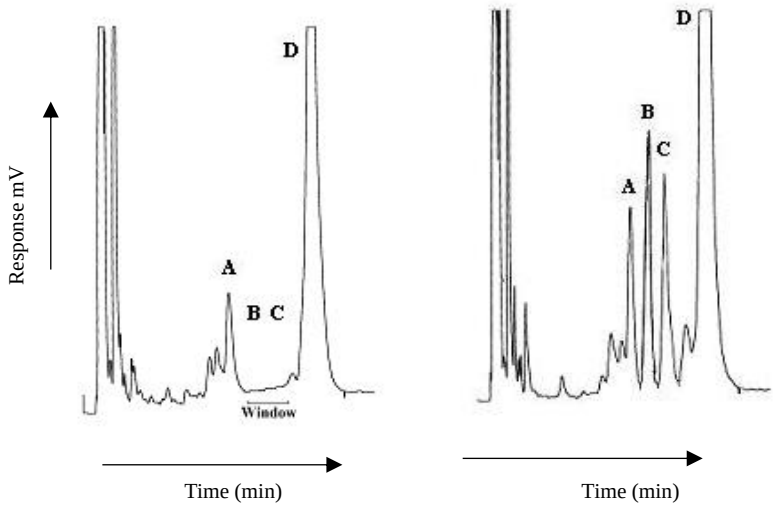
Commonly used adjuvant drugs, and salbutamol structural analogues were injected (as aqueous or dilute methanolic solutions unless otherwise specified) onto HPLC system to ascertain specificity and selectivity of the method for salbutamol and to find an appropriate Internal Standard (IS). These chemical entities included bamethane, fenoterol HBr, eformoterol, salmeterol, metapropterenol, synephrine (dissolved in dilute HCl), isoetharine, methoxyphenylamine HCl, pirbuterol, ±-phenylpropanolamine HCl (norephedrine), ephedrine HCl, isoprenaline sulphate, norphenylephrine (norfenefrin), de-oxyepinephrine, $_{DL}$-metanephrine, prenalterol, L-phenlyephrine HCl, clenbuterol,



i: Blank urine ii: 0.0-0.5 h urine sample iii: 0.5-24 h urine sample

Figure 4.3.1. Specimen chromatograms of un-hydrolysed blank and salbutamol containing urine samples – USAL METHOD

B=salbutamol, C=terbutaline, A & D=unknown peaks; samples of a male volunteer



i: Blank ii: 0.5-24 h Sample

Figure 4.3.2. Specimen chromatograms of hydrolysed blank and salbutamol containing urine sample – USALMET METHOD

B=salbutamol, C=terbutaline, A & D=unknown peaks; sample of a female volunteer.

methoxamine HCl (Vasoxine Inj. GlaxoWelcome), metaraminol tartrate (Aramine Inj. MSD), ipratropium bromide, pindolol, timolol, ±-metoprolol, nadolol, oxpremolol, atenolol, labetolol, aspartame, oxamniquine, caffeine, amphetamine, warfarin, naproxen, ketoprofen, fenbufen, acetanilide, sulindac, dopamine, tyramine HCl, beclomethasone dipropionate, estrone, ethyl paraben, methyl paraben and 4-benzyl biphenyl. Of the tested compounds none interfered with salbutamol. Terbutaline and bamethane were also fully resolved and were the only candidates for the internal standard (

Figure 4.3.3). However, the retention time of bamethane was very long (>45 min).

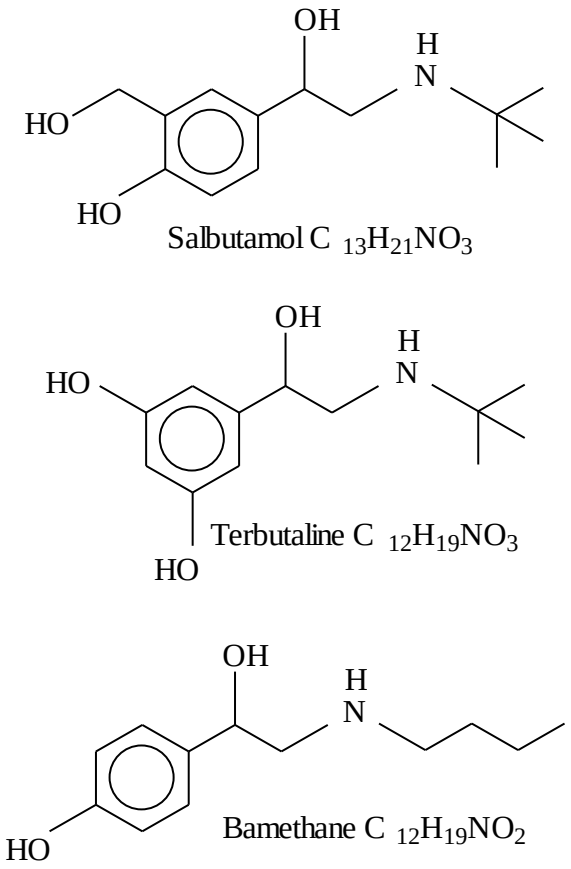


Figure 4.3.3. Structural formulae of salbutamol, terbutaline and bamethane.

4.3.1.4 Linearity and Range

The mean (n=6) regression equations using peak height ratios of salbutamol to terbutaline for aqueous standards (SAS), un-hydrolysed (USAL) and hydrolysed (USALMET) urine standards were y = 0.00488x + 0.00372, y = 0.00482x + 0.0000166

and y = 0.00448x+ 0.01455, respectively (Figure 4.3.4). The corresponding RSD of their slopes were 2.72, 1.56 and 1.68 % with respective standard deviation (SD) of the intercepts at 0.00299, 0.0058 and 0.0065. These slopes were not different from each other indicating that urine matrix had a minimal effect on the method (Brun and Veuthey, 1996). This could be due to effective clean-up of samples during extraction. The mean intercept (SD; 95% confidence interval) of USAL and USALMET at the lower limit of quantitation (LLOQ, 25 µg/L) was 0.05% (4.8; –0.022, 0.019) and 11.9 % (5.8; –0.010, 0.039), respectively.

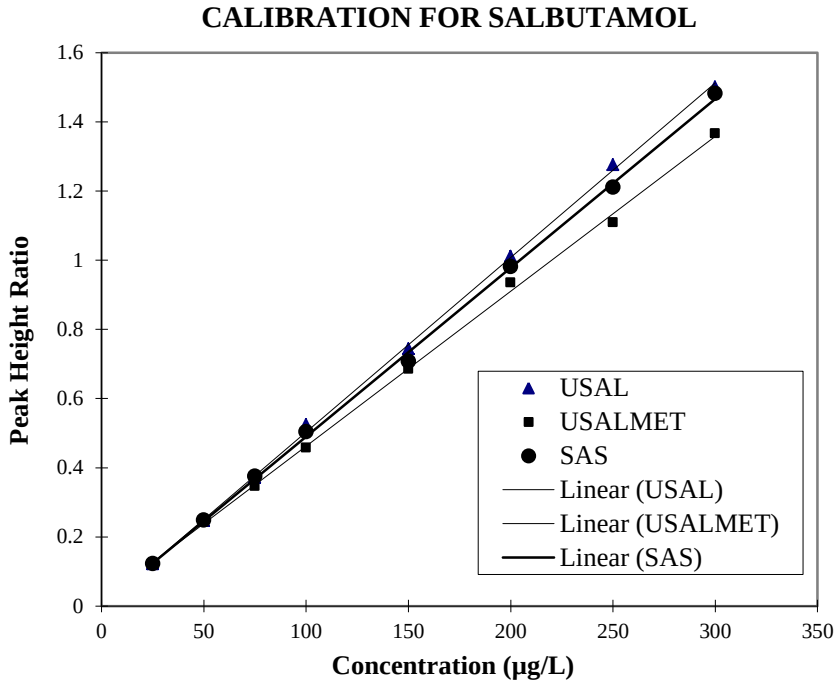


Figure 4.3.4. The Calibration lines of SAS, USAL and USALMET METHODS.

The regression lines were linear over the range 25 µg/L – 300 µg/L. The mean r^2 (RSD) values for SAS, USAL and USALMET were 0.9992 (0.1002%), 0.9983 (0.06%) and 0.9976 (0.202%), respectively. The calibrations were also found linear over the range 5 µg/L – 1000 µg/L. Since a smaller range is reported to tolerate larger deviations from linearity and make the method more rugged to non-linearity (Mulholland and Hibbert, 1997), a small concentration range with more close points spanned over the intended use of the method was selected.

4.3.1.5 Accuracy and Precision

The intra- and inter-day accuracy and precision (Table 4.3.1, Figure 4.3.5) for both USAL and USALMET urine standards were within acceptable limits of ±15% (FDA, 1994 & 2013; EMA, 2012). The results also depict the accuracy of the HPLC assay in precisely measuring unknown concentrations.

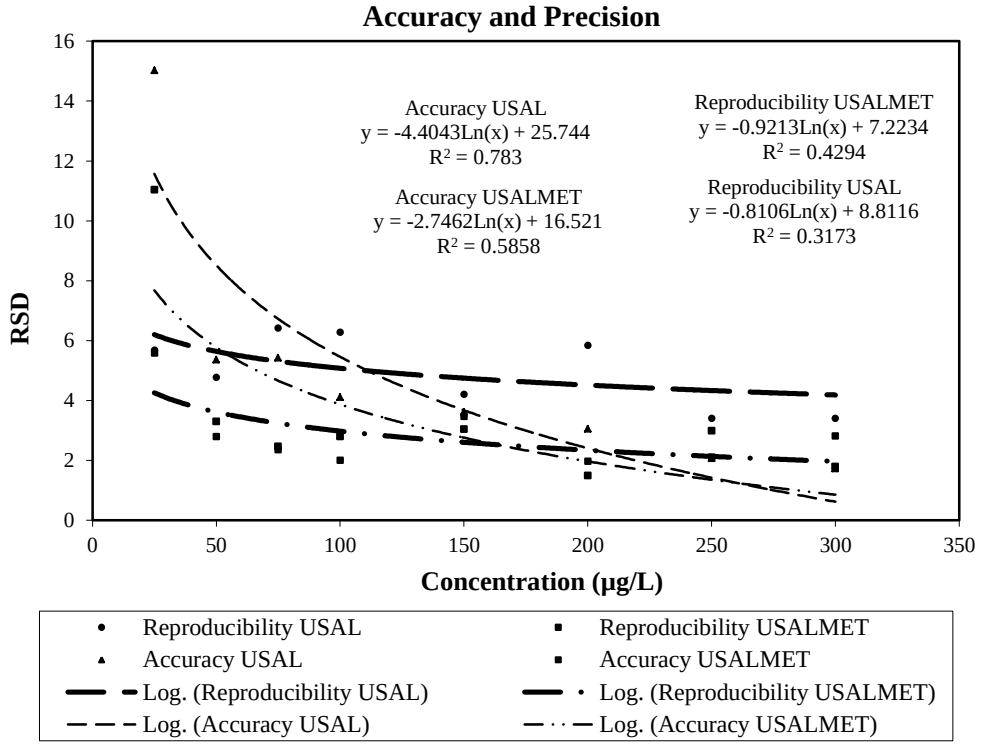


Figure 4.3.5. The graphic representation of reproducibility of accuracy and precision.

Light broken lines - RSD of recovered concentrations measured from individual regression curves. Bold broken lines - reproducibility of peak height ratio.

4.3.1.6 Limit of Detection and Quantitation (LOD and LOQ)

The calculated LOD for salbutamol aqueous (SAS), USAL (1 mL sample) and USALMET (1 mL sample) standards was 2.0, 4.0 and 4.8 µg/L, respectively, and the LOQ was 6.1, 12.1 and 14.6 µg/L. Repeated assays of three salbutamol concentrations, 5, 10 and 15 µg/L using the SAS method produced RSD values (n=10) of 4.1, 2.7 and 1.3 % respectively while the same concentrations using the USALMET METHOD gave RSD values (n=7) of 10.6, 3.9 and 3.9 %. The HPLC method is therefore highly

Table 4.3.1. Intra- and inter- day HPLC accuracy and precision (repeatability and reproducibility).

Nominal Concentration (µg/L)	USAL METHOD		USALMET METHOD		USAL METHOD	USALMET METHOD
	Mean Measured Concn* (RSD%)	Mean Bias (%)	Mean Measured Concn* (RSD%)	Mean Bias (%)		
a) Intra-Day Accuracy (n=3)					a) Intra-Day Repeatability (n=3) RSD (%)	
50	50.11 (5.72)	0.23	52.70 (0.92)	5.40	6.69	3.13
100	105.16 (4.62)	5.16	100.04 (3.24)	0.04	5.30	1.03
200	201.46 (3.96)	0.73	207.04 (1.66)	3.52	4.90	1.70
b) Inter-day Accuracy (n=6)					b) Inter-Day Reproducibility (n=6) RSD (%)	
25	24.30 (15.03)	-2.81	24.81 (11.04)	-0.78	5.68	5.59
50	49.03 (5.36)	-1.94	52.12 (3.31)	4.25	4.77	2.80
75	73.92 (5.43)	-1.44	74.57 (2.47)	-0.57	6.42	2.37
100	104.10 (4.12)	4.10	99.28 (2.80)	-0.73	6.28	2.01
150	147.64 (3.62)	-1.58	150.03 (3.05)	0.02	4.21	3.47
200	200.36 (3.05)	0.18	205.87 (1.50)	2.94	5.84	1.98
250	252.88 (2.07)	1.15	244.66 (2.99)	-2.14	3.41	2.11
300	297.47 (1.73)	-0.84	302.05 (1.80)	0.68	3.41	2.83

*Concn = Concentration

sensitive for quantifying salbutamol extracted in urine after inhalation. Specimen chromatograms of hydrolysed salbutamol urine standards 5, 10 and 25 µg/L containing terbutaline (500 µg/L) are shown in Figure 4.3.6.

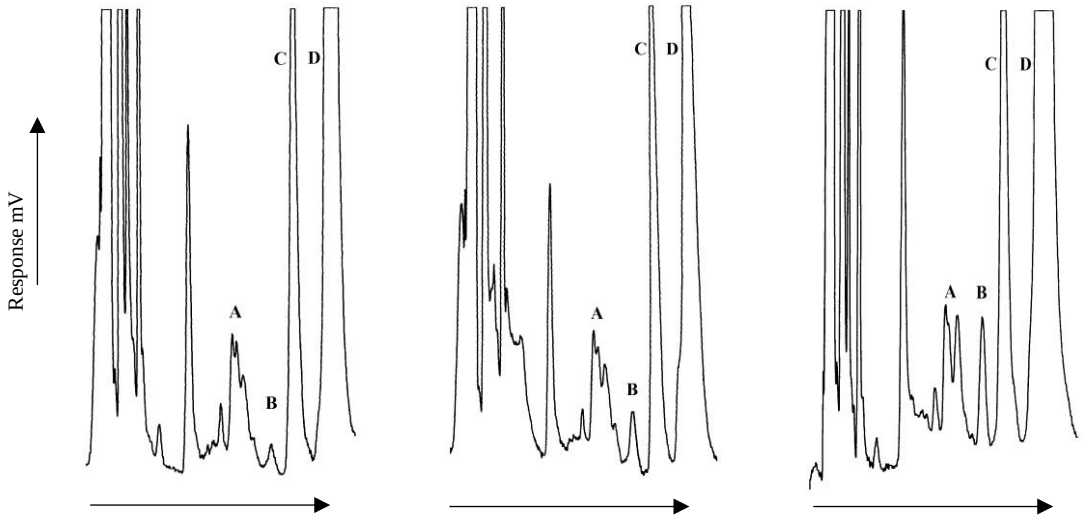


Figure 4.3.6. Specimen chromatograms of hydrolysed salbutamol urine standards (USALMET METHOD).

Salbutamol urine standards with terbutaline (i) 5 (ii) 10 and (iii) 25 µg/L

B=salbutamol, C=terbutaline, A & D= unknown peaks.

4.3.1.7 Robustness

The influence of different chromatographic parameters upon separation was evaluated by systematically varying the chromatographic conditions (ICH Q2(R1), 2005). Only one condition was changed while the others were kept constant. Slight variations in mobile phase constituents may change the width of the window for salbutamol and terbutaline which, however, remained fully resolved with respect to each other. The change in operating temperature by ±5°C did not affect resolution except back pressure. An increase in phosphate buffer molarity of up to 10 mM and of the ion-pair agent sodium dodecyl sulphate in the mobile phase up to 30 mM decreased the retention time with sharp peaks. However, this reduced resolution of salbutamol and terbutaline in hydrolysed urine samples and the width of the window squeezed by the unknown matrix peaks “A” and “D” (Figure 4.3.1 and Figure 4.3.2). Increasing molarity of the ion-pair agent also increased back-pressure.

4.3.2 SPE recovery, accuracy and precision

The results (obtained using peak heights of both un-hydrolysed and hydrolysed urine standards compared to direct aqueous injections) of intra- and inter-day SPE recovery, accuracy and precision are presented in Table 4.3.2. The pooled mean (RSD) intra-day percentage relative recoveries (%RR) of salbutamol using USAL and USALMET samples was 91.3 (0.05%) and 92.5 (2%) and the pooled mean precision RSD of % RR was 4.4 and 2.6 %, respectively. The pooled mean (RSD) inter-day %RR of salbutamol using USAL and USALMET samples was 90.8 (2.3%) and 91.5 (3.0%) and the pooled mean precision RSD of % RR was 2.9 and 3.3 %, respectively. The parallel %RR of terbutaline, added as the internal standard, was 90.2 (2.9%) and 96.4 (1.8%) with precision RSD of 4.9 and 3.9 %.

The USAL METHOD reported here is the mixed-mode SPE which is based on a control of the pH and thereby ionisation of the analyte(s) (Hennion, 1999; Fritz, 1999). The previously reported SPE method (Hindle and Chrystyn, 1992) lacked this control. The mean intra- and inter-day recoveries were within the accepted value of ±15% (Shah et al., 1992) indicating that the USAL METHOD was efficient, accurate and precise.

Extracting salbutamol from hydrolysed urine posed difficulties in controlling the pH, osmolarity and ionic strength of the analytes in the urine sample optimally with consequent variable and decreased recoveries of salbutamol and terbutaline. However, the pH of the acidified samples was raised and controlled in the region of pH ~7.0 with phosphate buffer (0.5 M, pH 13). The mean adjusted pH of 14 volunteers' hydrolysed samples was 6.80 (RSD 2.36; range 6.44-7.17; n=45). The mean adjusted pH of a female volunteer sample was 6.85 (RSD 1.97; range 6.58-7.06; n=24). The mean adjusted pH for 18 salbutamol urine standards was 6.73 (RSD 1.40, range 6.47-6.86). All these samples and standards contained terbutaline as internal standard. The pH control with KH_2PO_4 was precise and reproducible making the method robust.

Nevertheless, this strong buffering of hydrolysed urine samples resulted in high osmolarity and ionic density of the sample, which decreased recovery of salbutamol and terbutaline from HCX cartridges to less than 50% and 30%, respectively. Since polymeric cartridges are reported to possess a higher retaining capacity of analytes (Brun and Veuthey, 1996; Fritz, 1999; Masqué et al., 1998), Oasis HLB cartridges were used for extracting hydrolysed urine samples using the USALMET METHOD. Oasis

HLB were chosen instead of Oasis MCX (polymeric cationic) cartridges as the latter did not produce clean extracts. The USALMET METHOD gave reproducible recoveries of both salbutamol and terbutaline, which were comparable to USAL METHOD. This also indicates that the drying of eluates at 120ºC under a gentle stream of nitrogen in USALMET METHOD did not affect the integrity of the analytes. Besides, Mälkki-Laine et al. (1995) have reported virtually no decomposition of salbutamol on autoclaving for 20 min at 120ºC in solutions buffered at pH 3–5.

Table 4.3.2. Intra- and inter- day SPE accuracy and precision.

Nominal Concentration (µg/L)	% Relative Recovery (RR) of salbutamol			
	USAL METHOD		USALMET METHOD	
	% RR	RSD	% RR	RSD
a) Intra-day (n=3) SPE Recoveries of salbutamol				
50	91.32	4.72	94.09	2.03
100	91.39	3.40	90.47	4.32
200	91.30	5.21	92.83	1.47
b) Inter-day (n=6) SPE Recoveries of salbutamol				
25	93.15	4.32	96.02	4.76
50	90.99	3.50	92.95	2.66
75	87.77	2.22	87.86	3.43
100	88.89	3.19	89.49	3.05
150	92.70	3.26	93.95	2.32
200	89.30	3.65	92.22	1.34
250	93.37	2.18	90.04	3.89
300	90.37	1.02	89.80	4.87

Acid hydrolysis of salbutamol has been used to free it from its glucuronide conjugate (Evans et al., 1973; Hindle and Chrystyn, 1992; Forsdahl and Gmeiner, 2004). This is the first work where an internal standard has been used during acid hydrolysis to study the effect of any degradation of salbutamol itself in addition to freeing it from its glucuronide conjugate. Terbutaline, being a structural analogue, possesses similar physico-chemical properties as that of salbutamol (McDowall, 1989; The Merck Index, 2003; Ehrhardt et al., 2005). It was therefore added to the samples to reflect the stressful conditions salbutamol undergoes during acid hydrolysis. Forsdahl and Gmeiner (2004) have reported decomposition of salbutamol at 60ºC for 1 hour and could recover only 63% of intact salbutamol. This may be because they used 6 M HCl with the final

concentration of the acidified urine at 2 M. Evans et al. (1973) used 1 M HCl and reported that salbutamol remained unaffected by hydrolysis in a boiling water bath for 1 hour. The results of this work are in agreement with the findings of Evans et al. (1973). The samples were acidified to give a final concentration of 0.01 N HCl. The similarities and consistencies in recoveries of salbutamol and terbutaline indicate that both remained stable during acid hydrolysis for one hour at boiling temperature.

Although Oasis HLB cartridges could also be used for unchanged salbutamol when the samples were not hydrolysed they are more expensive than Confirm HCX cartridges. Also the preparation time when using Oasis HLB cartridges is longer because more steps are involved with the extraction. Thus on grounds of economy it is recommended that the Confirm HCX cartridges are used for unchanged salbutamol and when urine samples are hydrolysed for their salbutamol plus metabolite amounts then Oasis HLB cartridges are used.

4.3.3 Use of increased/multiple sample volume

The urine output of individuals is difficult to control over a set collection period. This is particularly important when patients are unable to inhale salbutamol dose correctly and completely from an MDI with resultant low levels excreted in urine. In such circumstances, a large output of urinary volume may necessitate the use of more than 1 mL of urine sample for the SPE to ensure consistent chromatographic response. The optimised and validated SPE conditions (based on the inhalation of two puffs of salbutamol (200 µg) by volunteers from an MDI) using 1–5 mL of the urine sample are shown in Table 4.3.3. Table 4.3.4 shows the results of the volunteer study using 1–5 mL of sample volume for the SPE. The individual and mean RSD values were within the acceptable limits (Shah et al., 1992) for both salbutamol and terbutaline which indicates that the extraction remained accurate and reproducible with the use of different sample volumes (1–5 mL). This may help in further increasing the sensitivity of HPLC by using multiple sample volume where appropriate. It was found that with higher urine volume in a given period the inherent urinary interferences were diluted which presented little concern for the resolution of salbutamol or terbutaline when more than 1 mL of sample was used for SPE to concentrate the sample.

Table 4.3.3. Sample preparation and pre-treatment methods before SPE.

Total Urine Output (mL) in the Sampling Period (hour)		Volume of Urine Sample to be taken (mL)	Volume of internal standard to be used (mL)	Pre-treatment of un-hydrolysed urine sample (USAL METHOD)				Pre-treatment of urine samples for hydrolysis and of hydrolysed urine (USALMET METHOD)				
				Molarity of Buffer KH_2PO_4, pH 7.0 (mM)	Volume of Buffer to add (mL)	Total Volume of Treated Sample (mL)	Final Molarity of Treated Sample (mL)	Normality of HCl to be added to sample (N)	Volume of HCl to be taken (mL)	Final Normality of Treated Sample (N)	Volume of KH_2PO_4, pH 13.0 to be used (mL)	Total Volume of Treated Sample (mL)
0-0.5	0.5-24											
Upto 75	Upto 750	1		30	2.0	4.0	15.00	0.10				11
75-150	750-1500	2		60	2.0	4.0	15.00	0.11				12
150-225	1500-2250	3	1	100	0.7	4.7	14.89	0.12	8	0.01	1	13
225-300	2250-3000	4		100	0.9	5.9	15.25	0.13				14
300-400	3000-4000	5		100	1.1	7.1	15.49	0.14				15

Table 4.3.4. Recovery and reproducibility of SPE (USALMET METHOD) with increasing volume of hydrolysed salbutamol urine standard and a volunteer's urine sample (USALMET24).

Sample Volume (mL)	Accuracy and Precision with increasing volume of hydrolysed salbutamol urine standards and samples (n=8)						
	Salbutamol urine standard (50 μg/L)				Volunteer's 0.5-24 hour urine sample (NK24)		
	Concn.* found (μg/L)	Recovered amount (μg)	Bias (%)	RSD (%)	Concn.* found (μg/L)	Recovered amount (μg)	RSD (%)
1	43.9	43.87	-12	5.93	114.6	189.55	6.63
2	88.2	44.09	-12	2.03	220.71	182.53	3.51
3	134	44.69	-11	3.36	326.44	179.98	3.10
4	183	45.68	-8.6	2.64	419.38	173.41	7.19
5	217	43.32	-13	2.89	492.68	162.98	5.23
Mean		44.33	-11.3	3.37		177.69	5.13
SD		0.90	1.8	1.51		10.05	1.82
RSD		2.03				5.66	

*Concn = Concentration; Salbutamol urine standard = 50 μg/L; Volunteer's urine sample = 0.5-24 hour

4.4 Stability Study

Analysis of a large number of samples over a prolonged period of time (overnight) makes it necessary that stability of the analyte(s) and internal standard in the carrier solvent for injection onto the automatable HPLC system be assured (Shah et al., 1992; ICH Q2(R1), 2005). Mälkki-Laine et al. (1995) have reported slowest decomposition of salbutamol in phosphate buffer (0.067 M, pH 5.2) over three days. Mälkki and Tammilehto (1990) also found maximum stability of salbutamol in aqueous solution at a pH of about 3.5 at 65°C. The results of this study are summarised in Table 4.4.1 and Table 4.4.2. This study shows that the mean (n=5) salbutamol and terbutaline recovered from the urine standards and the volunteer's sample (dissolved in the mobile phase) left at room temperature for up to 36 hours were consistent (>91% and ≥94%) and precise (RSD≤2% for both), respectively. The mean (n=10) salbutamol and terbutaline recovered from the urine standards and the volunteer's sample extracted concentrates frozen at –20°C for up to 40 days were also consistent (>88% for both) and precise (RSD≤4% and ≤3%), respectively. The mean (RSD) recovered amount of salbutamol over 0-36 hours (n=5) and over 40 days (n=10) from the 0.5-24 hour urine sample of the volunteer (KA24) was 210.5 μg (2.0%) and 187.6 μg (8.1%), respectively. The differences in recovery over the specified period were within the acceptable limits of ±15% (Shah et al., 1992). The mean (SD) percent change in measured concentration of salbutamol and terbutaline with subsequent injections (n=4) as compared to the 1st injection was ≤3% (3.1) and ≤2% (2.7), respectively. The mean (SD) percent change in measured concentration of salbutamol and terbutaline after freezing and defrosting (n=8) was ≤ −2% (1.2) and ≤ −3% (3.3), respectively. These variations may be considered due to inherent assay variability rather than a reflection of any instability of salbutamol or terbutaline. Also, the chromatograms of urine standards and the volunteer's sample did not show the appearance of any interfering or additional peaks over the test time-frame and no changes in chromatography were observed. Nevertheless, the peaks at position "A" (Figure 4.3.1, Figure 4.3.2 and Figure 4.3.5) grew in height over time in some of the SUS which, however, did not interfere with salbutamol peak at position "B". These stability studies indicate that salbutamol and terbutaline left at room temperature for up to 36 hours (dissolved in the mobile phase) and their extracted concentrates frozen at –20°C for up to 40 days did not show any significant variation of the measured concentration and recovery. The results of the two stability studies also demonstrate that the HPLC method is ruggedly robust.

Table 4.4.1. Stability indicating recovery of salbutamol and terbutaline.

Nominal Concn µg/L	Stability indicating Mean % Relative Recovery (%RR)							
	Stability in mobile phase over 0-36 hours at ambient temperature (n=5)				Stability of extracted concentrates after freezing at −20°C for up to 40 days (n=10)			
	Salbutamol		Terbutaline		Salbutamol		Terbutaline	
	% RR	RSD	%RR	RSD	% RR	RSD	%RR	RSD
50	90.82	5.04	94.61	2.45	88.99	3.65	89.39	2.20
100	93.38	3.36	97.13	1.83	84.67	4.51	89.68	1.42
200	91.46	2.13	94.41	2.51	90.72	1.92	90.43	4.14
KA24			93.67	2.90			85.76	5.57
Mean	91.89	3.51	94.96	2.42	88.12	3.36	88.82	3.33
SD	1.33	1.46	1.50	0.44	3.12	1.32	2.08	1.88
RSD	1.45		1.58		3.54		2.35	

Table 4.4.2. Stability indicating percent change in recovery of salbutamol and terbutaline.

Nominal Concn µg/L	Mean % Change (C) in Recovery with subsequent injections as compared to 1st injection (n=4)				Mean % Change (C) in Recovery after freezing and defrosting as compared to 1st day (n=8)			
	Salbutamol		Terbutaline		Salbutamol		Terbutaline	
	%C	SD	%C	SD	%C	SD	%C	SD
50	7.15	4.93	2.33	2.62	0.91	4.22	-3.17	1.86
100	2.60	3.72	2.34	1.78	-3.28	4.78	-2.10	1.19
200	2.63	2.12	1.11	2.87	-1.80	1.97	-4.97	3.81
KA24	-0.46	2.27	0.94	3.33	-2.39	3.98	-1.78	6.27
Mean	2.98	3.26	1.68	2.65	-1.87	3.71	-3.00	3.28
SD	3.13	1.33	0.76	0.65	1.98	1.22	1.44	2.28

4.5 Volunteer Study

The applicability of the method was demonstrated by determining urinary salbutamol concentration post inhalation using doses (two) equivalent to normal clinical practice (). In the past larger doses have been used to overcome assay sensitivity issues (Clark et al., 1996; Hindle and Chrystyn, 1992).

Figure 4.5.1 shows the average of the urinary concentrations for each of the 14 volunteers and the overall mean values (n=56) following the MDI inhalations (Part 1 Study). Similar values following inhalation from the MDIs with the co-administration of

oral charcoal (Part 2 Study) are shown in Figure 4.5.2. For each individual the USAL and USALMET data from the four study doses has been averaged. Following inhalation from the MDIs (Part 1 Study) the range of salbutamol concentrations was 22.1-501.3, 36.7-315.4 and 49.3-512.4 µg/L, respectively, for USAL0.5, USAL24 and USALMET24. Similar ranges for the MDI inhalations with the co-administration of oral charcoal (Part 2 Study) were 33.5-302.4, 12.1-94.8 and 37.0-195.7 µg/L. The range of urine volumes for all 0-0.5 and 0.5-24 hour collection periods was 15-580 and 370-2805 mL, respectively and the pH of all samples ranged from 4.7-8.

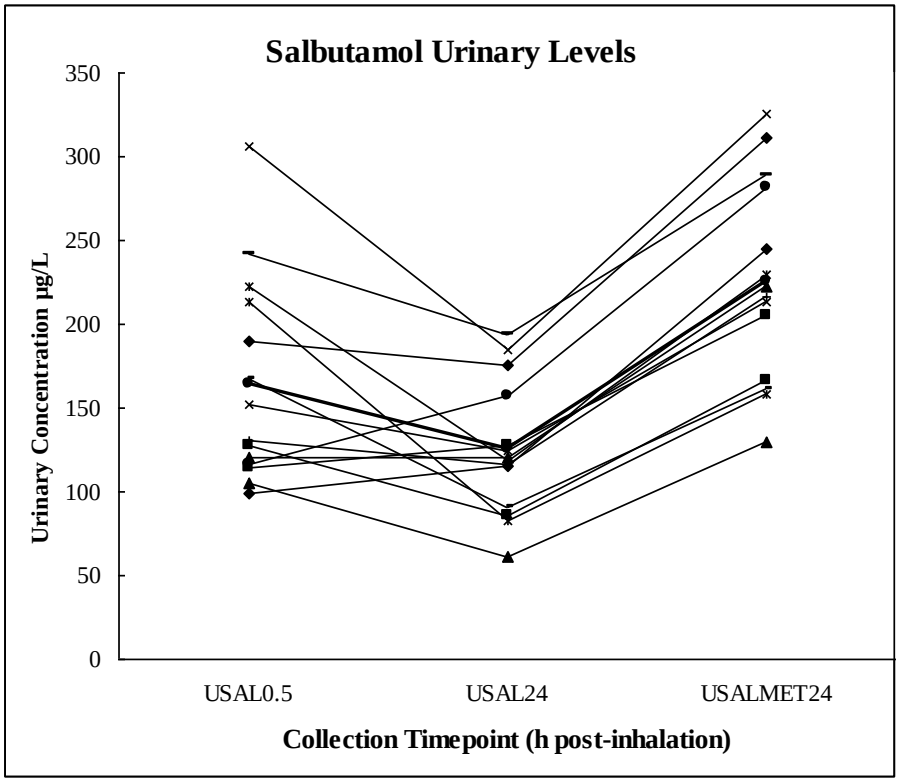


Figure 4.5.1. Mean urinary salbutamol concentration of individual volunteers following inhalation from MDIs (Part 1 Study).

The bold line indicates the mean of all the volunteers' samples.

The mean (n=56) amount of salbutamol dose (unchanged and metabolite fraction) recovered in the urine samples after the inhalation of 2 puffs (200 µg) from four different MDIs (Part 1 Study) and with the co-administration of oral charcoal (Part 2 Study), by the 14 volunteers, are shown in Table 4.5.1.

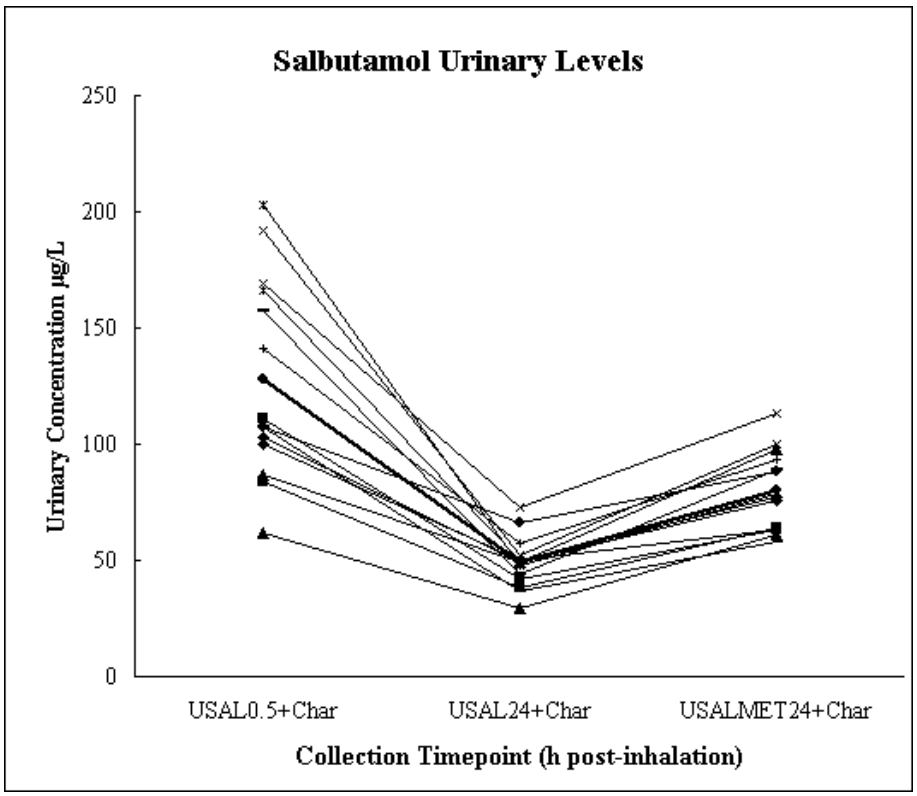


Figure 4.5.2. Mean urinary salbutamol concentration of individual volunteers following inhalation from MDIs (Part 2 Study).

The bold line indicates the mean of all the volunteers' samples.

The percentage of salbutamol dose recovered in urine in the first 0.5 hour (USAL0.5) post-inhalation from an MDI, without and with the co-administration of oral charcoal, is consistent with that reported earlier (Hindle and Chrystyn, 1992 & 1994; Hindle et al., 1997; Clark and Lipworth, 1996b; Silkstone et al., 2002a; Tomlinson et al., 2003). The amount of salbutamol excreted unchanged in urine in the first 0.5 hour after inhalation is believed to be mainly derived from the lung and is used as an index of relative bioavailability (Hindle and Chrystyn, 1992; Chrystyn, 2000 & 2001). The recovery of salbutamol dose in the first 30 min post-inhalation is considered to elicit the rapid bronchodilation and hence clinical effectiveness of an MDI as measured by spirometry. This index of relative bioavailability of salbutamol is used to identify the correlation of its pharmacokinetics and pharmacodynamics. The total amount of salbutamol and its metabolite excreted in the urine in the 24 hours post inhalation reflects the systemic delivery and is considered an indicator of the relative bioavailability of salbutamol to the body following an inhalation (Hindle and Chrystyn, 1992; Chrystyn, 2000 & 2001).

Table 4.5.1. Mean salbutamol dose recovered in the urine samples of healthy volunteers after inhaling two puffs of salbutamol from MDIs without and with charcoal co-administration.

	Urinary recovery of salbutamol dose (µg) in the given period (hour)				Total Recovered Dose (µg) 0.0-24 hour	Total Delivered (emitted) Dose (µg)
	USAL0.5[a]	USAL24[a]	Metabolite fraction 0.5-24[c]	USALMET24[b]		
MDI alone (Part 1 Study)						
Mean (SD)[d]	6.44 (3.36)	48.09 (17.06)	37.42 (15.89)	85.51 (21.7)	91.94 (22.43)	156.18 (9.92)
% of Nominal Dose (SD)	3.22 (1.68)	24.04 (8.53)	18.71 (7.94)	42.75 (10.85)	45.97 (11.21)	
Range	15.30-1.10	98.90-14.90	75.18-2.87	161.93-25.42		177.68-136.71
% of Recovered Dose (SD)	4.13 (2.16)	30.71 (10.45)	24.11 (10.47)	58.96 (14.49)		
MDI + Charcoal (Part 2 Study)						
Mean (SD)	6.57 (3.23)	19.99 (8.01)	12.82 (6.81)	32.81 (11.04)	39.38 (11.72)	151.75 (13.12)
% of Nominal Dose (SD)	3.28 (1.61)	10.00 (4.00)	6.41 (3.41)	16.41 (5.52)	19.69 (5.86)	
Range	16.93-2.53	38.43-6.83	31.31-3.62	67.38-17.88		172.67-121.72
% of Recovered Dose (SD)	17.38 (8.11)	50.71 (11.98)	31.91 (13.19)			

[a] assayed using the USAL METHOD and [b] assayed using the USALMET METHOD
[c] obtained from USALMET24 minus USAL24
[d] SD = Standard Deviation

In Part 1 Study, this amount was similar to that previously reported (Morgan, 1990; Hindle and Chrystyn, 1992). Hence approximately 46% of the nominal inhaled dose was delivered to the body via the pulmonary and gastro-intestinal routes. As a large proportion of the inhaled dose is swallowed (Pauwels et al., 1997), the salbutamol dose recovered in urine in 0.5–24 hour contains both unchanged and metabolised fractions. Since only unchanged salbutamol is effective in relieving bronchospasm, it is therefore necessary to ascertain the proportions of these fractions.

Charcoal blockage is used to separate absorption via the pulmonary and oral routes (Borgström and Nilsson, 1990; Silkstone et al., 2000; Ward et al., 2000) and to identify the total effective lung dose after inhalation (Chrystyn, 2001). Hence there was a difference between the amounts excreted in the urine in the 24 hour collections between the MDI inhalation without and with the co-administration of oral charcoal. The amount excreted over 24 hours with the co-administration of charcoal represents the amount that was deposited into the lungs and delivered to the systemic circulation. This was found to be 20% of the nominal dose. This value compares well with Olsson et al. (1996) and Chrystyn et al. (1997) and suggests that approximately 26% of the nominal dose was delivered to the systemic circulation and excreted in the urine.

The total inhaled bioavailable amount of salbutamol was 59% of the delivered (emitted) dose (lung + oral, Table 4.5.1) and 26% of this was due to pulmonary absorption. The undelivered dose varied from 21.91% (SD 4.96, range: 31.64-11.16%) to 24.12% (SD 6.56, range: 39.14-13.67%) for inhalation without and with charcoal respectively. Hindle et al. (1995) have reported similar results. About 20% of the delivered dose is un-accounted for in our studies. Of this, about 10% of the delivered dose drug could not be recovered during solid phase extraction (Table 4.3.2). The role of buccal absorption of salbutamol is not clear (Lipworth et al., 1989a; Lipworth, 1996; Spina et al., 1997; Valenzuela et al., 2001). The oesophageal absorption of salbutamol is still unknown. Therefore, the remaining 10% of the delivered dose would probably have excreted in the bile and/or in the faeces (Evans et al., 1973). Thus the mass balance of recovery of inhaled salbutamol from urine for 24 hour demonstrates the effectiveness of using urinary excretion as a measure of effective lung dose, relative bioavailability and total bioavailable dose.

4.6 Conclusion

The HPLC method was linear (over the range tested), precise, accurate and sensitive for determining salbutamol concentrations in human urine following the inhalation of normal doses. Two SPE methods for extracting salbutamol from un-hydrolysed and hydrolysed urine were efficient, reproducible and robust. A method using Confirm HCX cartridges is recommended for unchanged salbutamol and a different method using Oasis HLB is recommended for total salbutamol (salbutamol plus its metabolite). These methods were reliably applied to urinary pharmacokinetic studies using 14 volunteers after the inhalation of two 100 μg doses of salbutamol. The concentrations of unchanged and total salbutamol were within the sensitive range of the assay. This volunteer study revealed that about 20% of the nominal dose is delivered to the lungs and 46% to the systemic circulation following inhalations from a metered dose inhaler.

5 Chapter 5: *In-Vitro* and *In-Vivo* Equivalence of Salbutamol HFA MDIs

5.1 Overview

In-vitro studies on MDIs provide critical information on their dose delivery characteristics and aerodynamic particle size distribution (APSD) profiles. Pharmacopoeias recommend Andersen Cascade Impactor (ACI) to obtain this information (BP, 2005; USP28-NF23, 2005; Ph. Eur., 2011).

Physical properties of inhaled drug molecules, their APSD and aerodynamic particle diameter define *in-vivo* lung deposition (Hickey et al., 1996; Harrison et al., 1997; Howarth, 2001; Guo et al., 2008). These characteristics of aerosol particles influence as to where they will deposit in the human respiratory tract (HRT) (Chrystyn, 2000 & 2001; Mobley and Hochhaus, 2001) to produce therapeutic and systemic effects (Pritchard, 2001; Weda et al., 2004; Usmani et al., 2005).

With the phase-out of CFC propellants under the Montreal Protocol agreement (UNEP, 2017), replacement salbutamol metered dose inhalers (MDIs) containing HFA propellant are since then available. Development, transition, and comparative *in-vitro* studies on salbutamol HFA MDIs were carried out by manufacturers of MDIs (Ross and Gabrio, 1999; Cripps et al., 2000; McCabe et al., 2012) and add-on devices (Mitchell et al., 1999; Hatley et al., 2014; Sanders and Bruin, 2015; Johnson et al., 2016). However, these studies employed 5 to 10 puffs. It has been reported that firing multiple puffs of salbutamol MDI results in the loading effect in ACI measurements and leads to aerosol particle entrainment (Nasr and Allgire, 1995; Nasr et al., 1997). In addition, multiple puffs mask the emitted dose variations. Consequently, the resultant APSD does not represent the true particle size distribution. Hence, in the current study, a clinically relevant dose (2 puffs) has been used. Further, the present *in-vitro* studies are complemented by *in-vivo* pharmacokinetic studies.

This chapter is organised into separate sections comprising of *in-vitro* and *in-vivo* equivalence studies on salbutamol HFA MDIs.

5.2 *In-Vitro* Equivalence of Salbutamol HFA MDIs-Aerodynamic Particle size Characterisation

The aim of this study is to determine APSD using ACI to investigate *in-vitro* equivalence of salbutamol HFA MDIs.

5.2.1 Materials and Methods

5.2.1.1 Materials and Equipments

Details provided in Section 3.3.1.1 (Chapter 3 Methodology).

5.2.1.2 Test MDIs

Ventolin Evohaler™ (Evo), Airomir™ (Airo) and Salamol™(Sala).

5.2.2 Study Design

Protocols 3.3.1 and 3.3.2 (Sections 3.3.2.3 & 3.3.2.4; Chapter 3) describe the study design as per pharmacopoeial requirements. In brief, one puff of a randomly selected primed salbutamol HFA MDI was discharged into ACI operated at a flow rate of 28.3 L/min for 8.5 seconds to allow 4L of air to pass through it. The second puff was similarly discharged after 30 seconds. The amount of salbutamol deposited on ACI components and stages was quantified using validated HPLC method (Chapter 4).

5.2.3 Results: *In-Vitro* Equivalence of Salbutamol HFA MDIs

The mean amount (n=5) of two actuations of salbutamol deposited on various components and stages of ACI recovered from Evo, Airo and Sala are shown in Table 5.2.1. Their individual run data is provided in Appendices 5.2.3.1 to 5.2.3.3, respectively. Figure 5.2.1 and Figure 5.2.2 respectively show complete APSD profiles and cumulative particle size deposition of the three MDIs. Summaries and comparisons of various CQAs are provided in Table 5.2.2 to Table 5.2.6 and Figure 5.2.3 & Figure 5.2.4. Results of statistical analysis and *in-vitro* equivalence of MDI performance metrics are given in Table 5.2.7 to Table 5.2.9.

The mass balance and total emitted dose (TED) of Ventolin Evohaler, Airomir and Salamol were within 5% and 25% of labelled metered dose (100 μg) per actuation (Table 5.2.1). This confirms system suitability. Hence, the results generated are valid and accurate (Christopher et al., 2003).

Table 5.2.1. APSD of Salbutamol HFA MDIs.

Identity	Ventolin Evohaler			Airomir			Salamol		
	µg	SD	RSD	µg	SD	RSD	µg	SD	RSD
MDI Canister Valve	5.0	0.5	9.9	19.6	2.7	13.8	20.0	2.2	10.8
MDI Actuator	36.2	2.9	8.1	21.0	1.9	9.1	28.8	5.3	18.4
ACI IP (Throat)	88.8	7.4	8.3	69.7	2.1	3.0	80.1	3.1	3.8
ACI S-0	2.1	0.2	8.8	3.5	0.8	24.3	2.7	0.6	22.4
ACI S-1	2.8	0.3	12.5	4.0	1.1	27.5	3.8	0.8	21.3
ACI S-2	4.6	0.6	12.1	5.8	1.4	23.7	4.1	0.5	11.9
ACI S-3	18.4	2.2	12.1	25.1	3.4	13.5	19.0	2.5	13.4
ACI S-4	35.2	2.6	7.3	35.7	1.8	4.9	35.8	4.3	12.1
ACI S-5	19.9	1.9	9.7	21.0	1.4	6.8	24.0	3.8	15.8
ACI S-6	3.4	0.3	8.3	5.2	0.5	8.7	5.8	1.0	16.9
ACI S-7	0.6	0.1	13.2	2.1	0.0	1.5	2.3	0.5	21.5
ACI Filter	0.9	0.2	19.9	2.3	0.4	17.8	2.6	0.8	31.7
Total Recovery (μg)	217.8	8.9	4.1	214.9	3.4	1.6	228.9	7.4	3.2
% Recovery[a]	108.9	4.4	4.1	107.5	1.7	1.6	114.4	3.7	3.2
Mass Balance[b] (μg)	212.8	8.6	4.0	195.3	3.5	1.8	208.8	6.2	3.0
% Recovery	106.4	4.3	4.0	97.7	1.8	1.8	104.4	3.1	3.0
TED[c] (μg)	176.6	7.6	4.3	174.3	1.7	1.0	180.0	5.2	2.9
% TED	88.3	3.8	4.3	87.2	0.8	1.0	90.0	2.6	2.9

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 μg per puff.

b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

c = TED (Total Emitted Dose Ex-Actuator). Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve and Actuator (mouth piece).

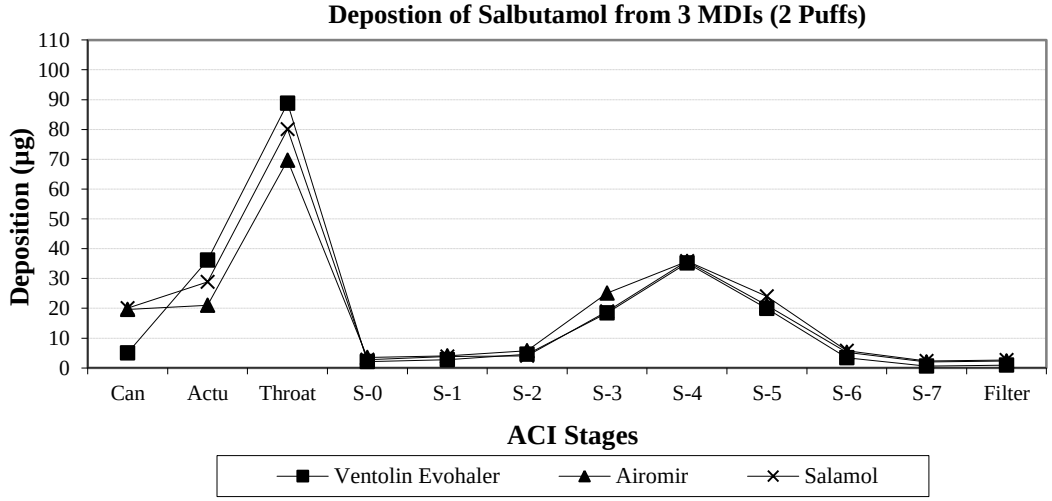


Figure 5.2.1. Mean APSD profiles of salbutamol HFA MDIs.

Can = MDI Canister; Actu = MDI Actuator

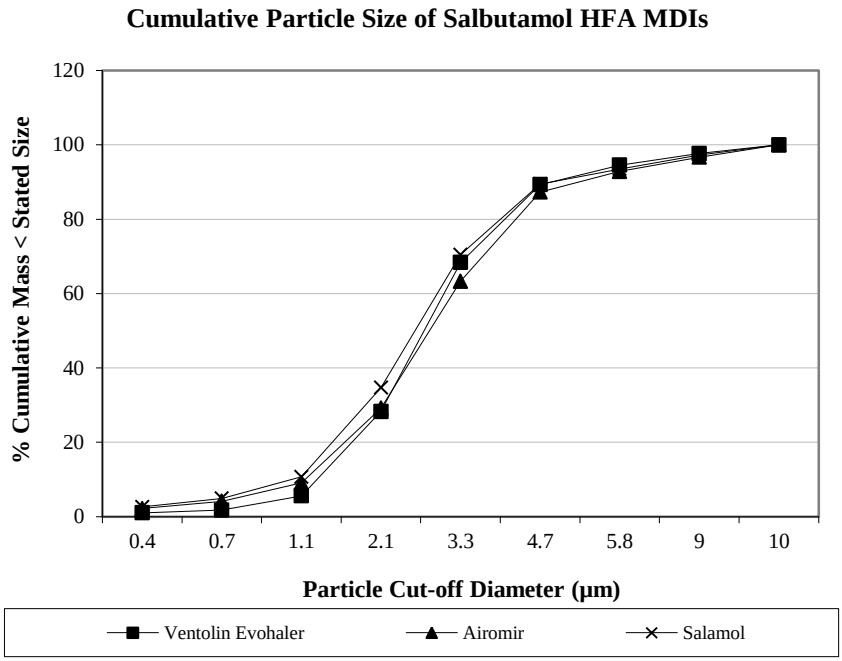


Figure 5.2.2. Mean percent cumulative particle size deposition profiles of salbutamol HFA MDIs.

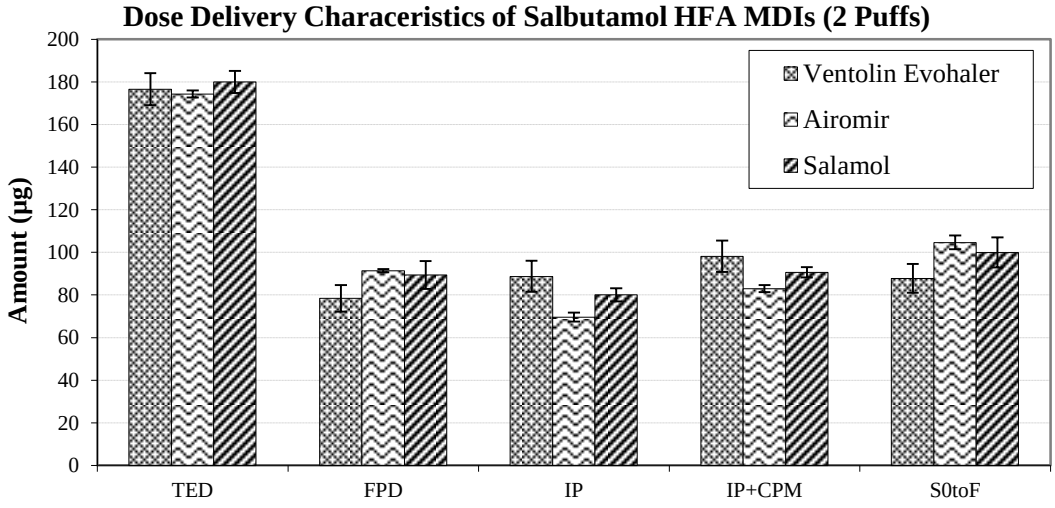


Figure 5.2.3. Dose delivery characteristics of salbutamol HFA MDIs.

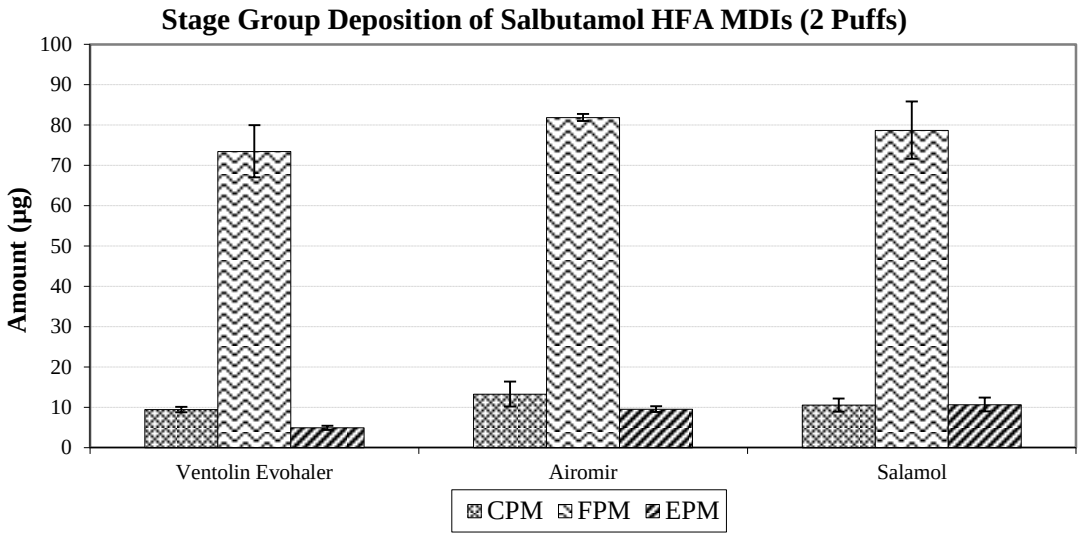


Figure 5.2.4. Stage group deposition of salbutamol HFA MDIs.

TED of three salbutamol MDIs was both statistically similar and *in-vitro* equivalent (Table 5.2.2 & Table 5.2.7).

IP deposition was neither statistically similar nor *in-vitro* equivalent amongst the three MDIs (Table 5.2.2 & Table 5.2.9). Impactor mass (S0toF) was both statistically similar and *in-vitro* equivalent between Airomir and Salamol only. With Ventolin Evohaler, TED was evenly distributed between IP and ACI plates (S0toF). This IP deposition was higher than both Airomir and Salamol. With Airomir, ~60% of TED entered ACI assembly (S0toF); with Salamol, TED was ~11% more than its IP deposition.

S0toF deposition was statistically similar and *in-vitro* equivalent only between Airomir and Salamol. Their S0toF as %TED was also *in-vitro* equivalent, albeit with a statistically significant difference.

FPD and %FPF were significantly lower for Ventolin Evohaler as compared to Airomir and Salamol (Table 5.2.3, Table 5.2.4 & Table 5.2.7) which rendered it *in-vitro* inequivalent to them. On the other hand, Airomir and Salamol MDIs had both statistically similar and *in-vitro* equivalent FPD and %FPF.

Table 5.2.2. Dose delivery and deposition in ACI of salbutamol HFA MDIs.

Treatment Method	TED		IP		IP+CPM		S0toF		IP (%TED)		IP+CPM (%TED)		S0toF (%TED)	
	µg	SD	µg	SD	µg	SD	µg	SD	%	SD	%	SD	%	SD
Ventolin*	176.63	7.55	88.78	7.36	98.21	7.40	87.85	6.77	50.25	3.48	55.59	3.31	49.75	3.48
Airomir	174.33	1.69	69.68	2.10	82.94	1.66	104.65	3.24	39.98	1.42	47.58	0.57	60.02	1.42
Salamol	180.03	5.21	80.07	3.07	90.62	2.39	99.96	7.11	44.52	2.54	50.38	2.36	55.48	2.54

* Ventolin Evohaler

Table 5.2.3. FPD, Stage groups, MMAD and GSD of salbutamol HFA MDIs.

Treatment Method	FPD		FPM		EPM		CPM		MMAD		GSD	
	µg	SD	µg	SD	µg	SD	µg	SD	µm	SD		SD
Ventolin*	78.42	6.28	73.49	6.46	4.93	0.51	9.43	0.68	2.68	0.03	1.56	0.02
Airomir	91.38	0.75	81.87	0.89	9.51	0.72	13.27	3.08	2.77	0.13	1.60	0.06
Salamol	89.41	6.58	78.71	7.10	10.70	1.74	10.55	1.60	2.56	0.07	1.62	0.05

* Ventolin Evohaler

Table 5.2.4. FPD and stage groups as %TED of salbutamol HFA MDIs.

Treatment Method	FPF (%TED)		FPM (%TED)		EPM (%TED)		CPM (%TED)	
	%	SD	%	SD	%	SD	%	SD
Ventolin*	44.41	3.31	41.62	3.49	2.79	0.26	5.34	0.28
Airomir	52.42	0.57	46.96	0.51	5.46	0.43	7.60	1.71
Salamol	49.62	2.36	43.67	2.84	5.95	1.05	5.86	0.88

* Ventolin Evohaler

FPM deposition shows that only Airomir and Salamol met *in-vitro* equivalence criterion although the three MDIs were statistically equivalent in this respect (Table 5.2.3 & Table 5.2.9). On the other hand, CPM and EPM depositions did not meet *in-vitro* equivalence criterion. Statistical similarities of CPM were found between Ventolin Evohaler Vs Salamol and Airomir Vs Salamol. However, EPM deposition showed statistical similarities only between Airomir Vs Salamol.

Table 5.2.5. FPD and stage groups as %S0toF of salbutamol HFA MDIs.

Treatment Method	**FPD (%S0toF)**		**FPM (%S0toF)**		**EPM (%S0toF)**		**CPM (%S0toF)**	
	%	SD	%	SD	%	SD	%	SD
Ventolin*	89.25	0.60	83.59	1.41	5.65	0.87	10.75	0.60
Airomir	87.38	2.59	78.28	2.22	9.10	0.80	12.62	2.59
Salamol	89.44	1.52	78.70	3.37	10.74	2.02	10.56	1.52

* Ventolin Evohaler

Table 5.2.6. FPD and S0toF delivery efficiency of salbutamol HFA MDIs.

Treatment Method	**FPD / IP**		**FPD / IP+CPM**		**S0toF / IP**	
	Ratio	SD	Ratio	SD	Ratio	SD
Ventolin*	0.89	0.12	0.80	0.10	1.00	0.13
Airomir	1.31	0.04	1.10	0.03	1.50	0.09
Salamol	1.12	0.12	0.99	0.09	1.25	0.13

* Ventolin Evohaler

In summary, the three MDIs showed *in-vitro* equivalent TED, MMAD and GSD. FPD and %FPF (%TED) of Ventolin Evohaler significantly differed from both Airomir and Salamol and was not *in-vitro* equivalent. On the other hand, Airomir and Salamol were *in-vitro* equivalent with respect to TED, FPD, FPF (%TED), FPM, S0toF, FPF (% S0toF), FPM (% S0toF), S0toF (%TED), MMAD and GSD.

Table 5.2.7. *In-Vitro* Equivalence of TED, FPD and S0toF of salbutamol HFA MDIs.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p* value	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
TED (µg)	Ventolin Evohaler	Airomir	1.01	0.96	1.07	1.000	Yes	1.15	-4.37	6.68	1.000	Yes
		Salamol	0.98	0.93	1.04	1.000	Yes	-1.70	-7.22	3.83	1.000	Yes
	Airomir	Salamol	0.97	0.92	1.02	0.880	Yes	-2.85	-8.37	2.68	0.843	Yes
FPD (µg)	Ventolin Evohaler	Airomir	0.86	0.77	0.95	0.006	No	-6.48	-11.22	-1.74	0.005	No
		Salamol	0.88	0.79	0.97	0.023	No	-5.49	-10.23	-0.75	0.018	No
	Airomir	Salamol	1.02	0.92	1.14	1.000	Yes	0.99	-3.76	5.73	1.000	Yes
FPF (%TED)	Ventolin Evohaler	Airomir	0.85	0.78	0.92	<0.0001	No	-.080	-0.12	-0.04	0.0002	No
		Salamol	0.89	0.82	0.97	0.014	No	-.052	-0.09	-0.01	0.011	No
	Airomir	Salamol	1.06	0.97	1.15	0.545	Yes	0.03	-0.01	0.07	0.367	Yes
S0toF (µg)	Ventolin Evohaler	Airomir	0.84	0.76	0.93	0.001	No	-8.40	-13.61	-3.18	0.001	No
		Salamol	0.88	0.79	0.97	0.020	No	-6.05	-11.27	-0.84	0.018	No
	Airomir	Salamol	1.05	0.95	1.16	1.000	Yes	2.34	-2.87	7.56	1.000	Yes
%S0toF (%TED)	Ventolin Evohaler	Airomir	0.83	0.77	0.89	<0.0001	No	-10.27	-14.72	-5.82	<0.0001	No
		Salamol	0.90	0.83	0.97	0.009	No	-5.73	-10.18	-1.27	0.008	No
	Airomir	Salamol	1.08	1.00	1.17	0.087	Yes	4.55	0.09	9.00	0.044	No
FPF (%S0toF)	Ventolin Evohaler	Airomir	1.02	0.99	1.05	0.547	Yes	1.86	-1.31	5.04	0.577	Yes
		Salamol	1.00	0.97	1.03	1.000	Yes	-0.19	-3.36	2.98	1.000	Yes
	Airomir	Salamol	0.98	0.95	1.01	0.400	Yes	-2.06	-5.23	1.12	0.414	Yes

CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 5.2.8. *In-Vitro* Equivalence of MMAD and GSD of salbutamol HFA MDIs.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p* value	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
MMAD (µm)	Ventolin Evohaler	Airomir	0.97	0.91	1.03	1.000	Yes	-0.09	-0.27	0.09	0.951	Yes
		Salamol	1.05	0.99	1.12	0.308	Yes	0.13	-0.05	0.30	0.292	Yes
	Airomir	Salamol	1.08	1.02	1.15	0.019	Yes	0.21	0.04	0.39	0.014	No
GSD	Ventolin Evohaler	Airomir	0.98	0.93	1.04	1.000	Yes	-0.03	-0.13	0.07	1.000	Yes
		Salamol	0.97	0.91	1.02	0.635	Yes	-0.06	-0.16	0.05	0.680	Yes
	Airomir	Salamol	0.98	0.93	1.04	1.000	Yes	-0.03	-0.13	0.08	1.000	Yes

CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 5.2.9. *In-Vitro* Equivalence of IP and stage group depositions of salbutamol HFA MDIs.

Stage Grouping	Multiple Comparisons		Mean Ratio	90% CI		*p* value	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
IP (Throat)	Ventolin Evohaler	Airomir	1.27	1.16	1.40	<0.0001	No	9.55	5.13	13.97	<0.0001	No
		Salamol	1.11	1.01	1.21	0.063	No	4.35	-0.07	8.78	0.055	Yes
	Airomir	Salamol	0.87	0.79	0.96	0.006	No	-5.20	-9.62	-0.77	0.017	No
Group 1 (CPM) (S0+S1+S2)	Ventolin Evohaler	Airomir	0.73	0.55	0.95	0.038	No	-1.92	-3.67	-0.17	0.027	No
		Salamol	0.90	0.69	1.18	1.000	No	-0.56	-2.31	1.19	1.000	Yes
	Airomir	Salamol	1.24	0.94	1.63	0.302	No	1.36	-0.39	3.11	0.197	Yes
Group 2 (FPM) (S3+S4+S5)	Ventolin Evohaler	Airomir	0.89	0.79	1.01	0.156	No	-4.19	-9.13	0.76	0.129	Yes
		Salamol	0.93	0.83	1.05	0.900	No	-2.61	-7.55	2.33	0.791	Yes
	Airomir	Salamol	1.04	0.92	1.18	1.000	Yes	1.58	-3.37	6.52	1.000	Yes
Group 3 (EPM) (S6+S7+F)	Ventolin Evohaler	Airomir	0.52	0.42	0.64	<0.0001	No	-2.29	-3.40	-1.18	<0.0001	No
		Salamol	0.46	0.38	0.57	<0.0001	No	-2.88	-3.99	-1.77	<0.0001	No
	Airomir	Salamol	0.90	0.73	1.11	1.000	No	-0.59	-1.70	0.52	0.773	Yes

CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

5.2.4 Discussion: *In-Vitro* Equivalence of Salbutamol HFA MDIs

Discussion is organised into separate sections and follows underneath.

5.2.4.1 Total Emitted Dose (TED)

TED demonstrates *in-vitro* equivalence of Ventolin Evohaler, Airomir and Salamol. TED for Ventolin Evohaler is comparable to but higher than that reported by McCabe et al. (2012), Nagel et al. (2011) and Cripps et al. (2000) with corresponding ratios of ~0.97, ~0.87 and ~0.93 (Table 2.5.1). TED reported by Johnson et al. (2016) shows a ratio of ~0.82 (normalised for metered dose) and indicates larger difference.

TED of Airomir is similar to that reported by Dubus et al. (2001) and Mitchell et al. (1999) but higher than that of Johnson et al. (2016); their respective ratios are ~0.98, ~0.93 and ~0.72 (normalised for metered dose of Proventil HFA) (Table 2.5.2).

TED of ProAir HFA reported by Johnson et al. (2016), McCabe et al. (2012) and von Hollen et al. (2011a & b & 2012) is similar to TED reported here for Salamol, with respective ratios of ~0.98 (normalised for metered dose), ~1.03 and ~1.01 (Table 2.5.3).

5.2.4.2 Induction Port (Throat) Deposition

IP deposition was in decreasing rank order of Ventolin Evohaler > Salamol > Airomir. Johnson et al. (2016) also reported similar trend for Ventolin HFA, ProAir HFA and Proventil HFA.

Ventolin Evohaler had significantly higher IP deposition than Airomir and Salamol. This is more likely associated with its inherent product characteristics, vis-à-vis: emission of forceful high velocity spray with resultant longer travelling, shorter life plume (Ross and Gabrio, 1999; Stein, 2008; Brambilla et al., 2011; McCabe et al., 2012). Ventolin Evohaler, which contains HFA propellant only and no excipient (Table 2.2.1), was developed to simulate product performance characteristics of Ventolin CFC it replaced; hence spray patterns and plume characteristics were reproduced to give patient the same feel of throat impaction (Cripps et al., 2000). On the other hand, in addition to HFA propellant, Airomir and Salamol contain ethanol as co-solvent while Airomir also contains surfactant oleic acid. Addition of ethanol in MDI formulation reduces spray velocity, impact force, plume geometry and prolongs plume life (Gabrio et al., 1999; Ross and Gabrio, 1999; Smyth, 2003; Leach, 2005; Stein and Myrdal,

2006; Hess, 2008; Stein, 2008; McCabe et al., 2012; Ivey et al., 2015). Hence, these formulation constituents in Airomir and Salamol may have contributed to relatively lower IP deposition and more proportion of their TED entered ACI assembly (S0toF) than Ventolin Evohaler. Besides, the three MDIs have differences in device design such as orifice diameter, fill volume, valve structure and components (Ross and Gabrio, 1999; Gabrio et al., 1999; Stein, 2008; Brambilla et al., 2011; McCabe et al., 2012). Therefore, these differences in their formulation and device design may have resulted in significantly different IP deposition.

IP deposition reported in this thesis for Ventolin Evohaler lies between that reported by Cripps et al. (2000) and Johnson et al. (2016). Their respective IP deposition ratios are ~0.88 and ~1.15 (normalised for metered dose) and suggest similarity with results reported here although their results differed significantly from each other (ratio ~0.76).

For Airomir, respective ratios of IP depositions reported by Johnson et al. (2016) and Ross and Gabrio (1999) to those reported here are ~0.82 (normalised for metered dose) and ~1.26 and depict significant differences. IP deposition reported in this thesis lies within the range of their results. Again, results of these investigators significantly differ from each other (ratio ~0.65). Also, Mitchell et al. (1999) reported that ~53% of labelled unit dose (90 µg) was retained in IP, which is higher than that reported in this thesis (~40% of 90 µg).

IP deposition reported by Johnson et al. (2016) for ProAir HFA is smaller than that reported in this thesis for Salamol (ratio ~0.81) (normalised for metered dose).

In addition to formulation factors, the differences in IP deposition could be due to continuous evaporation of propellant (and ethanol) in ACI at varying rates while emitted dose is traversing through it (Stein and Myrdal, 2004; Myrdal et al., 2004; Stein, 2008). This evaporation rate is also affected by the environment, i.e., temperature and humidity (Labiris and Dolovich, 2003). This is evident from the variability in IP deposition observed within the five runs reported in this thesis (Table 5.2.1).

The differences in IP deposition highlight inconsistency of reported results by investigators and complicate safety assessment of swallowed dose for a given MDI. This unpredictability may be confounded by patient factors.

5.2.4.3 Impactor Mass (S0toF)

Impactor deposition mimics the dose that would be inhaled and available for clinical effects in HRT beyond throat. S0toF results showed significant differences of Ventolin Evohaler with both Airomir and Salamol resulting in their *in-vitro* inequivalence to the former. These differences are due to variability of IP deposition with consequent differences in amounts reaching into impactor (S0toF) (Borgström et al., 2006; Cheng et al., 2015). Similar trend was observed when S0toF was assessed as %TED. Also, S0toF to IP deposition ratios were in decreasing rank order of Airomir > Salamol > Ventolin Evohaler. The highest S0toF Vs IP ratio of Airomir indicates its TED had more proportion of impactor mass which in turn resulted in higher FPD. These findings suggest that differing amounts of TED from these MDIs will reach into HRT beyond throat, which may have clinical implications.

On the other hand, impactor mass was both statistically similar and *in-vitro* equivalent between Airomir and Salamol, albeit significantly lower IP deposition of Airomir. This *in-vitro* similarity between them is more likely due to relatively higher TED of Salamol, and consequently more dose entered the impactor thereby off-setting the effects of higher IP deposition. This was evident from their S0toF (%TED) *in-vitro* equivalence.

5.2.4.4 Fine Particle Dose (FPD)

FPD (and FPM) of Ventolin Evohaler did not meet *in-vitro* equivalence criteria when compared with Airomir and Salamol. Results suggest that the latter two MDIs were more efficient than the former MDI in producing FPD which was in decreasing rank order of Airomir > Salamol > Ventolin Evohaler. Johnson et al. (2016) reported significantly different FPD amongst Ventolin HFA, Proventil HFA and ProAir HFA which was in the decreasing rank order of ProAir HFA > Proventil HFA > Ventolin HFA. However, these investigators found larger differences; the ratios of Ventolin HFA Vs Proventil HFA and Vs ProAir HFA were 1.92 and 3.08, respectively. This is at variance to FPD results reported here where the ratios for Ventolin Evohaler Vs Airomir and Vs Salamol are 0.86 and 0.88. Besides, their reported FPD ratio between ProAir HFA and Proventil HFA is 1.61 which is again significantly different than reported here for Airomir Vs Salamol. The latter two MDIs were indeed found *in-vitro* equivalent in this study with a FPD ratio of 1.02. Hence, differences in FPD between these two studies may have origins in varying and smaller TED and IP deposition (Sections 5.4.2.1 & 5.2.4.2). McCabe et al. (2012) compared ProAir HFA with Ventolin HFA.

The ratio of 2.04 obtained for their reported FPD indicates significant difference. This is in conflict with the ratio of 0.88 reported for Salamol to Ventolin Evohaler in this thesis.

Ratios of FPD reported by Johnson et al. (2016), McCabe et al. (2012), Nagel et al. (2011) and Cripps et al. (2000) to the FPD of this study for Ventolin Evohaler are respectively ~0.44 (normalised for metered dose), ~0.67, ~ 0.89 and ~0.95. FPD reported by Johnson et al. (2016) and McCabe et al. (2012) are significantly lower while those reported by Nagel et al. (2011) and Cripps et al. (2000) are comparable to that reported here. In contrast, Sanders and Bruin (2015) have reported a significantly higher FPD (ratio ~ 1.41). Coppolo et al. (2005) reported FPD for generic Ratio-Salbutamol HFA MDI which is significantly lower than that reported in this thesis (ratio ~ 0.71) despite having been manufactured under GSK agreement with Ratio-Pharma (Patented Medicine Prices Review Board, 2011).

For Airomir, FPD reported by Ross and Gabrio (1999), Mitchell et al. (1999) and Dubus et al. (2001) is more or less similar to that reported in this thesis having respective ratios of ~0.85, ~1.09 and ~1.11 (< 5.8 *u*m). However, FPD reported by Johnson et al. (2016) for Proventil HFA is lower than that reported here having a ratio of ~0.73 (normalised for metered dose).

Higher FPD has been reported for ProAir HFA by Johnson et al. (2016), McCabe et al. (2012) and von Hollen et al. (2011a & b & 2012). Their respective results have ratios of ~1.20 (normalised for metered dose), ~1.18 and ~1.29 as compared to Salamol. This comparison indicates that FPD obtained by these investigators is similar between them but higher than that reported in this thesis.

These comparisons with other investigators reveal that it is not uncommon to have differing FPD amongst HFA MDIs. The varying FPD results reported in this thesis for Ventolin Evohaler, Airomir and Salamol reflect on this tendency. However, the differences amongst these FPD results reported in this thesis are of lesser magnitude than those reported by Johnson et al. (2016) and McCabe et al. (2012). Nevertheless, it is highly likely that these differing results amongst HFA MDIs are due to differences in their formulations and device design. Such varied and conflicting results will have implications for prescribers and are likely to confuse clinicians in decision making.

5.2.4.5 FPD to IP Ratio

Pritchard (2001) highlighted that the amount of inhaled drug delivery to lung in relation to throat deposition should be optimal to keep a balance between therapeutic effects and systemic side effects. He suggested that an ideal ratio of lung to total systemic exposure should be one. Depositions on IP and on stages 3 to 7 and filter (FPD) respectively represent depositions in throat and lungs in humans (Table 3.3.2). Ratios of FPD to IP deposition were in decreasing rank order of Airomir > Salamol > Ventolin Evohaler. Further, similar trend was observed with ratios of FPD (respirable dose) to IP+CPM (non-respirable dose) depositions. These ratios suggest that Airomir may deliver higher proportion of respirable dose. However, with Salamol these ratios are closer to unity and therefore it may be ideally placed HFA MDI with respect to a better benefit to risk ratio.

5.2.4.6 Fine Particle Fraction Percentage (%FPF)

FPF as %TED was in decreasing rank order of Airomir > Salamol > Ventolin Evohaler. Johnson et al. (2016) found this order to be as ProAir HFA > Proventil HFA > Ventolin HFA. McCabe et al. (2012) also reported higher %FPF for ProAir HFA than Ventolin HFA. Thus, both Airomir and Salamol had higher %FPF. This trend is more likely due to higher IP deposition of Ventolin Evohaler as compared to the other two MDIs. As a consequence, relatively lower TED entered ACI assembly (S0toF) and resulted in lower FPD. The role of formulation in IP deposition has been discussed earlier (Section 5.2.4.2).

Ratios of %FPF reported by Johnson et al. (2016), McCabe et al. (2012), Nagel et al. (2011) and Cripps et al. (2000) to that reported in this thesis are ~0.54, ~0.74, ~ 1.02 and ~0.89, respectively. %FPF of former two studies is significantly different while for the latter two studies, it is similar to that reported here.

For Airomir, ratios of %FPF reported by Johnson et al. (2016) (Proventil HFA), Dubus et al. (2001), Ross and Gabrio (1999) and Mitchell et al. (1999) to that reported in this thesis are ~1.02, ~1.21, ~0.91 and ~0.96, respectively. These ratios suggest similarity of their results with the current study, except for Dubus et al. (2001). However, Dubus et al. (2001) calculated %FPF with TED obtained from Emitted Dose Uniformity (EDU) tests rather than TED obtained from ACI testing.

For ProAir HFA, ratios of %FPF reported by Johnson et al. (2016), McCabe et al. (2012) and von Hollen et al. (2011a & 2012) to the results reported here for Salamol are

respectively ~1.23, ~1.14 and ~1.28. These ratios show higher %FPF was found by others.

It is evident that %FPF reported here for Airomir compares favourably with those reported by others. However, conflicting results were found with some studies for Ventolin Evohaler and Salamol. The magnitude of these differences in %FPF indicate the scale of variations noted amongst different studies for a given MDI as pointed out in Sections 5.2.4.2 and 5.2.4.4.

5.2.4.7 Fine Particle Fraction as percent of Impactor Mass (FPF as %S0toF)

This parameter reflects on the proportion of respirable dose that would reach to HRT beyond throat. Even though differing amounts of TED reached impactor, yet the proportion of FPD as %S0toF (87% to 89%) was statistically similar and *in-vitro* equivalent amongst the three MDIs. This finding is the outcome of off-set effect of the relative, inconsistent and disproportionate variability of S0toF and FPD. This may mask the actual differences in FPD delivery efficiency of these MDIs. The variability of these two metrics has been discussed earlier (Sections 5.2.4.3 and 5.2.4.4).

5.2.4.8 MMAD and GSD

Irrespective of their formulation differences, the three MDIs produced aerosol particles which met *in-vitro* equivalence criteria with respect to their MMAD and GSD; with only statistically significant difference observed between Airomir Vs Salamol for MMAD. Their MMAD range of > 2.5 µm to < 2.8 µm represents the desired particle size range (FPM), and therefore each of the three MDIs is expected to deliver inhaled dose to bronchi and bronchioles to elicit bronchodilation (Pritchard, 2001; Howarth, 2001; Clark, 2012). Their GSD range of 1.56 to 1.62 approximates monodispersity. This suggests that their aerosolised drug particles had a similar and narrower particle size distribution around their respective MMADs (Figure 5.2.2). Since FPD and FPM were in order of decreasing amounts of Airomir > Salamol > Ventolin Evohaler, this may affect dose delivery to desired regions of HRT. Airomir may be expected to deliver relatively more dose to bronchi and bronchioles than both Salamol and Ventolin Evohaler because of having more proportion of particles within the respirable range. This may have clinical implications.

Pritchard (2001) pinpointed that aerosolised particles with MMAD <2.5 µm are more likely to be exhaled if patients do not hold their breath. MMADs reported by other

investigators for Ventolin HFA, Proventil HFA and ProAir HFA are <2.5 µm (Ross and Gabrio, 1999; Cripps et al., 2000; von Hollen et al., 2011a & b & 2012; McCabe et al., 2012; Hatley et al., 2014; Johnson et al., 2016;), and therefore more likely to be found in the region at the borderline between FPM and EPM. These results are in contrast to those reported here where MMAD size of > 2.5 µm suggests a larger proportion of particles in FPM range. Since the amount of salbutamol that reaches to lungs has been linked to its effects (Melchor et al., 1993; Zanen et al., 1994 & 1996; Weda et al., 2004; Usmani et al., 2005), therefore, results reported in this thesis may be more reflective of clinical effects than those reported by these investigators.

GSDs reported by other investigators as compared to those found in this study are higher for Ventolin Evohaler (≥ 1.8) (McCabe et al., 2012; Johnson et al., 2016), similar for Airomir (~ 1.6) (Johnson et al., 2016) and lower for ProAir HFA (Vs Salamol) (≤ 1.6) (McCabe et al., 2012; Johnson et al., 2016). These variations and similarities may be reflected in clinical effects when viewed in conjunction with APSD, FPM and MMAD.

5.2.4.9 Role of Actuator Design

Airomir and Salamol were *in-vitro* equivalent with respect to the CQAs vis-à-vis: TED, FPD, FPM, %FPF, S0toF, MMAD and GSD. Also, their CPM and EPM were statistically similar. This is perhaps understandable since both MDIs contain ethanol as co-solvent (Table 2.2.1). However, it is perhaps surprising given that their actuator mouthpiece design is different, i.e., round Vs rectangular (oval). For Airomir, it has been postulated that its round shaped actuator mouthpiece creates wider space in mouth (oral cavity) thereby providing the dispensed dose more room for evaporation into finer particles which are then inhaled (Ross and Gabrio, 1999). The round actuator mouthpiece of Airomir and its slow and soft puff of emitted dose are hypothesized to act in synergy thereby reducing throat deposition and enhancing lung delivery. This hypothesis holds good for Airomir with respect to IP deposition reported in present study which is significantly lower than that found for Salamol. Johnson et al. (2016) have also reported slightly lower IP deposition for Proventil HFA than ProAir HFA. However, given that Salamol performed similarly *in-vitro* with respect to the above mentioned CQAs of an MDI performance, the significance of this hypothesis is debatable. The data of current project could not support the possible link of actuator mouthpiece shape of Airomir to other MDI performance metrics mentioned above.

Interestingly, Johnson et al. (2016) reported lower TED and FPD for Proventil HFA than ProAir HFA. Also, Asmavent MDI (Neolab / Fannin (UK) Limited) was granted generic approval by Medicines and Healthcare Products Regulatory Agency (MHRA) and considered equivalent to Airomir (PL 08137/0130; 3rd July 2009). Asmavent has similar formulation ingredients to that of Airomir but has a rectangular mouthpiece design (Fannin PIL, 2014; Fannin SmPCs, 2017). Hence, based on present *in-vitro* results and other published work, it can be argued that the shape of the actuator did not have effect on CQAs of Airomir and Salamol.

5.2.4.10 Stage Groups

ACI stage pooling into groups in this study is consistent with earlier reports (Guo et al., 2008 & 2013; de Boer et al., 2015). Collective deposition on these stage groups highlights their relevance to various regions of HRT (Pritchard, 2001, Weda et al., 2004; Usmani et al., 2005).

Stage group comparison of the three MDIs revealed *in-vitro* inequivalence. The only exception was *in-vitro* equivalence between Airomir and Salamol for stage group 2 (FPM). These dissimilarities are likely due to differences in their formulations and device design with consequences in dose delivery characteristics (Sections 5.2.4.2 and 5.2.4.4). These findings concur with those of de Boer et al. (2015) for four ICS/LABA DPI formulations in different devices. They reported marked differences in APSDs and amounts of submicron (diameter < 1 µm) and micron (1–3 µm) particle mass fractions. However, they also found most mass fractions of particles in the range of 3–5 µm were similar which supports findings for FPM in this thesis. Hence, different formulations with different inhaler device design may not produce similar APSD profiles and may have consequences for clinical effects.

Thus, findings in this study for stage group comparison suggest that the three HFA MDIs may not be interchangeable. This is reflected in the directive of MHRA to prescribers to specify brand of MDI (Chrystyn and Price, 2009). Further, US Food and Drug Administration (FDA) categorised Ventolin HFA, Proventil HFA and ProAir HFA as therapeutically inequivalent (code BX) (FDA Orange Book online). Also, MHRA did not allow using generic name '*Salbutamol Inhaler'* for Asmavent CFC-free MDI (generic of Airomir), arguing that the product must be identifiable by a brand name (PL 08137/0130; 3rd July 2009). The findings of current study complement these regulatory injunctions.

5.2.4.11 Which Criteria for *in-vitro* equivalence?

Inhaled bronchodilator therapy is meant to relieve bronchospasm such as caused by acute asthma (GINA, 2017). The delivery of inhaled dose to lungs is dependent on particle size composition of dispensed dose from an MDI. Particles that have aerodynamic size less than 5 µm have the greatest chance to deposit in lungs (Chrystyn, 1997) and bring about bronchodilatation.

Clinical studies in chronic stable asthma have revealed a correlation between total dose of salbutamol deposited in the lung and improvement in FEV_1 (Zainudin et al., 1990). Melchor et al. (1993) found a significant relationship between the amount of salbutamol deposited in lungs and its bronchodilatory effects in healthy subjects and patients. APSD profile (S0toF) of salbutamol MDI reported in their manuscript reflected on clinical effects. In studies comparing the effects of particle size of inhaled salbutamol in asthma (Zanen et al., 1994) and COPD (Zanen et al., 1996) patients, the bronchodilator response was greater when salbutamol particles of 2.8 µm size were inhaled than when the same patients inhaled particles sized 1.5 µm and 5 µm. These investigators also showed that bronchodilatory response in asthma patients was dependent on the amount of drug reaching lungs. Total lung deposition was also found to be linked with fine particle dose (Olsson et al., 1996). Lung deposition is optimal when aerosol cloud contains majority of particles within size range of 2-5 µm (Chrystyn, 1997). Weda et al. (2004) demonstrated that safety of three salbutamol formulations inhaled from Novolizer DPI was correlated to particles deposited on impactor stages in FPD range. However, it has also been shown that monodisperse salbutamol particles of 6 µm deposit in the airways and produce similar bronchodilatory response when compared to particles of 3 µm (Usmani et al., 2003 & 2005; Usmani, 2008). Hence, findings of these studies indicate that particles of more than one size bring about bronchodilatation. Most MDIs are polydisperse (Nagao et al., 2005) and produce particles over a wide range of size and therefore it is highly likely that a range of particles sizes are involved in therapeutic effects (Pritchard, 2001). Recently, it has been shown in a PK study using charcoal block approach that systemic availability of salbutamol was related to lung deposition and that adverse events were correlated to systemic levels (Moore et al., 2017). These researchers also suggested that FPM was related to PK profile for the same inhaler device. These studies clearly suggest that FPD is the key CQA of salbutamol MDI which can provide comparative information on both *in-vitro* and *in-vivo* performance.

Individual stage-wise comparisons could have value for quality assurance, product monitoring and in research and development of new inhaler products. However, differently formulated MDI products with a different device design will have a differing APSD profile which would result in high variability between individual stage depositions. This is evident from the results of current study. Also, at present no link between individual stage deposition and expected deposition sites in lungs has been established (García-Arieta, 2014). Hence stage-wise comparison would be less predictive of *in-vivo* behaviour of inhaled drug besides being impractical for application. However, comparisons based on stage groups are considered anticipative of *in-vivo* bioequivalence (García-Arieta, 2014). Even then it is highly unlikely to clearly link a stage group with efficacy and safety (Usmani et al., 2003 & 2005; Usmani, 2008).

Salbutamol MDI is prescribed for rapid relief of airways obstruction which is achieved by its local lung deposition. Hence, efficacy to provide quick relief of asthma symptoms is the key desired objective while the incidence of side effects is of low importance. Efficacy of salbutamol MDI is related to its FPD that is deposited in lungs. Therefore, comparisons of individual stages or group of stages which reflect on side effects of a salbutamol MDI become less important. Besides, salbutamol related side effects from MDIs are rare at the recommended dose (PILs of Ventolin Evohaler, Airomir and Salamol). In addition, a significant portion of TED that would deposit in IP and on stages 0 to 2 (CPM) is removed when a spacer is used with an MDI (Barry and O'Callaghan, 1997; Mitchell et al., 1999; Coppolo et al., 2006; Mazhar and Chrystyn, 2008; Hall et al., 2011; Hatley et al., 2014; Oliveira et al., 2015 & 2016; Johnson et al., 2016) (also see Chapters 6, 7 & 8). Consequently, delivered dose from an MDI attached to a spacer mainly contains particles that constitute FPD. Since a comparison of an MDI used alone and with attached spacer is a regulatory pre-requisite where a specific spacer is to be used with a specific MDI, this comparison is achieved by comparing their FPDs (EMA, 2006 & 2009). Thus, the comparison of MDIs with respect to their FPDs would be a meaningful, practical and effective criterion to establish *in-vitro* equivalence. Hence, *in-vitro* equivalence has been concluded based on comparable FPD.

The range of stages to represent FPD would depend on the type of inhaler product and objective of treatment. This choice of stages has varied amongst researchers. Cripps et al. (1999) defined FPD for salbutamol to consist of stages 2-6 while Peyron et al. (2005)

compared fine particle mass (FPM) of salmeterol on stages 3 to 5 (particles between 1.1 µm to 4.7 um). Usmani (2008) and co-workers (2003 & 2005) have shown that salbutamol particle size of 6 µm also possesses bronchodilatory effects. However, in this thesis FPD has been defined as per recommendations of EMA guideline (2006 & 2009), i.e., particles size < 5 µm which effectively means deposition on ACI stages 3 to 7 and back-up filter when operated at 28.3 L/min. This FPD specification has been widely reported (Ross and Gabrio, 1999; Mitchell et al., 1999; Rau et al., 2006; Coppolo et al., 2006; Guo et al., 2008; Laube et al., 2011; Hall et al., 2011; von Hollen et al., 2011 and 2012; Hatley et al., 2014; Sanders and Bruin, 2015; Sandell and Mitchell, 2015; Johnson et al., 2016; Hillyer et al., 2018).

5.2.4.12 Summary of Discussion

In this *in-vitro* study, APSD profiles obtained from ACI analysis of Ventolin Evohaler, Airomir and Salamol were compared in accordance with EMA guidelines (2006 & 2009).

The recommendations of EMA guideline have been assessed with respect to their practicality for determining *in-vitro* equivalence using ACI. Comparisons based on individual stages were not performed due to high variability between individual stages of the three MDIs. Instead, comparisons based on grouped stages were carried out along with the traditional (and regulatory) approach of comparing FPD as the principal criterion. Stages were grouped as CPM, FPM and EPM. These comparisons showed significant differences amongst pooled stages. IP deposition also differed significantly. Therefore, the three MDIs were deemed not *in-vitro* equivalent. *In-vitro* equivalence was observed only between Airomir and Salamol for FPM. Similar pattern of *in-vitro* equivalence was observed when FPD was used as the main comparative criterion. Since similar results were obtained with the two comparative approaches, it is therefore deemed that comparison based on FPD represented a more practical approach to assess differing formulations of MDIs containing salbutamol. Besides, this approach is more predictive of *in-vivo* effects (Section 5.2.4.11).

TED, MMAD and GSD were *in-vitro* equivalent amongst the three MDIs. However, S0toF, FPD and %FPF were only *in-vitro* equivalent between Airomir and Salamol. This similarity between these two MDIs is more likely due to the presence of ethanol in their formulations. Therefore, based on similar FPD, these two MDIs can be considered *in-vitro* equivalent.

5.2.5 Conclusions: Salbutamol HFA MDI *In-Vitro* Study

Based on *in-vitro* significant differences in FPD, Ventolin Evohaler should not be considered an equivalent treatment to either Airomir or Salamol and therefore should not be substituted with either of them. On the other hand, Airomir and Salamol had *in-vitro* similar FPD. However, on the basis of EMA (2006 & 2009) criteria, none of these MDI was found *in-vitro* equivalent and caution must be exercised for substituting one for the other.

APSD profiles of the three MDIs assessed as stage groups were not *in-vitro* equivalent. This is likely due to differences in their formulation and device design with consequent differences in drug delivery characteristics.

Since *in-vitro* equivalence studies on the three salbutamol MDIs could not be entirely demonstrated based on stage groups, this necessitates conducting a comparative *in-vivo* (PK) study as the next step forward.

5.3 *In-Vivo* Equivalence of Salbutamol HFA MDIs-Urinary Pharmacokinetic Studies

The objectives of these PK studies are to demonstrate *in-vivo* equivalence of Ventolin Evohaler, Airomir and Salamol by comparing their relative lung and total systemic bioavailability in healthy subjects. This study also estimates lung deposition of inhaled salbutamol by preventing its gastrointestinal (GI) absorption of the swallowed fraction of drug using activated charcoal.

5.3.1 Study Design

Details of the study design have been provided in Section 3.4.5 (Chapter 3 Methodology). In brief, thirteen trained healthy volunteers (7 females) took part in this six way crossed-over two part open study. In Part 1 Study, on separate study days (one week apart), each volunteer inhaled 2 puffs in two separate manoeuvres separated by 30 seconds from randomly selected salbutamol MDI. The volunteers exhaled to residual volume prior to actuation, then took a slow deep inhalation over 5–10 seconds, followed by a 10 second breath hold (Section 3.4.4). This procedure was repeated for second actuation. In Part 2 Study, each volunteer repeated this study with the concurrent administration of activated charcoal by swallowing 100 mL of charcoal slurry immediately before and after two inhalations (Mazhar and Chrystyn, 2008). Volunteers swished charcoal slurry in the mouth before swallowing all of it.

Urine samples were collected 0.5 hour before and after inhalation and thereon total urine was pooled for 24 hours. Volume and pH of all urine samples were recorded. All urine samples were stored at –20°C till extracted and assayed.

5.3.2 Sample Analysis

Urine and aqueous samples were processed and analysed using validated HPLC methods described in Chapter 4.

5.3.3 Statistical Analysis

Statistical analysis performed as per Section 3.4.7 (Chapter 3).

5.3.4 Results: *In-Vivo* Equivalence of Salbutamol HFA MDIs

The mean (SD) age, height, weight and BMI of volunteers was 31.2 (7.6) years, 1.68 (0.07) meters, 64.9 (10.8) Kg and 22.9 (2.4) Kg/m^2, respectively; 54% were females and 46% were Caucasians (Table 5.3.1 and Appendix 5.3.4.1). The pH of all urine samples was between 4.5 and 6.5. Hence, pH-dependent renal clearance of salbutamol was unlikely (Hindle and Chrystyn, 1992). All volunteers inhaled the dose correctly.

Table 5.3.1. Demographic Characteristics of Volunteers.

Characteristics	**Overall (n = 13)**	**Male (n=6)**	**Female (n=7)**
Age, mean (SD)	31.2 (7.6)	36.8 (7.6)	26.3 (2.5)
(range), years	(23-48)	(25-48)	(23-31)
Sex, n (%)	13	6 (46%)	7 (54%)
Height, mean (SD)	1.68 (0.07)	1.73 (0.07)	1.64 (0.05)
(range), m	(1.57-1.82)	(1.64-1.82)	(1.57-1.70)
Weight, mean (SD)	64.9 (10.9)	70.7 (10.3)	59.9 (9.2)
(range), Kg	(48-82)	(54-82)	(48-72)
BMI, mean (SD)	22.9 (2.4)	23.6 (2.4)	22.3 (2.4)
(range), Kg/m^2	(18.3-27.1)	(20.1-27.1)	(18.3-25.0)
Race, n (%)			
Caucasian	6 (46%)	1 (16.7%)	5 (71.4%)
Asian	7 (54%)	5 (83.3%)	2 (28.6%)

BMI = Body Mass Index

Summaries of the mean amounts of salbutamol recovered at 0.5h (USAL0.5) and 24h (USAL24) post-dose (without and with charcoal block) are provided in Table 5.3.2 and shown in Figure 5.3.1 to Figure 5.3.5 (see Appendices 5.3.4.2 to 5.3.4.7 for individual data). The table and figures also depict salbutamol as free (USAL24Pre), free and sulphate conjugated salbutamol (USAL24Post) and metabolised (USALMET) moieties excreted during 0.5h to 24h period. Figure 5.3.6 shows comparative salbutamol urinary recovery profiles obtained post-inhalation without and with charcoal ingestion. These recoveries of salbutamol as % nominal, % delivered and % recovered dose are given in Table 5.3.3 to Table 5.3.5. Table 5.3.6 and Table 5.3.7 provide data on *in-vivo* equivalence and statistical significance of the three salbutamol MDIs in Parts 1 and 2 studies, respectively.

The mean ratios of the three MDIs did not meet *in-vivo* equivalence criteria for any of the parameters studied in the two parts. In Part 1 Study (without charcoal ingestion),

most of these parameters were statistically similar amongst the three MDIs (Table 5.3.6). However, the amount of free salbutamol excreted in 0.5h-24h (USAL24Pre) was statistically significantly different amongst them. Nevertheless, total free and conjugated salbutamol excreted during this period (USAL24Post) was only significantly different between Ventolin Evohaler and Airomir.

In Part 2 Study (with charcoal ingestion), only USAL0.5 and USALMET were statistically similar amongst the three MDIs (Table 5.3.7). USAL24Pre was statistically similar between Ventolin Evohaler Vs Airomir and Airomir Vs Salamol; the latter pair was also statistically similar for USAL24Post.

Table 5.3.2. Mean salbutamol excreted in urine post-inhalation from MDIs.

Treatment Method (n = 13)	**USAL0.5**		**USAL24†**		**USAL24Pre**		**USAL24Post**		**USALMET**	
	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD
Part 1 Study (without charcoal blockade)										
Ventolin*	5.7	1.9	100.7	15.7	58.4	18.3	95.0	16.6	36.6	15.6
Airomir	7.1	3.3	84.2	28.1	42.1	14.0	77.1	27.0	35.0	18.2
Salamol	6.7	3.2	94.4	19.4	42.9	11.2	87.7	18.41	44.8	12.9
Part 2 Study (with charcoal blockade)										
Ventolin*	5.3	2.5	29.8	5.3	14.3	5.3	24.5	4.8	10.2	5.1
Airomir	6.7	3.9	40.1	12.7	20.9	7.8	33.4	11.7	12.5	7.3
Salamol	7.2	3.4	50.2	13.1	27.2	11.6	43.0	13.5	15.7	8.9

* Ventolin Evohaler; † TRD = USAL24; SD = Standard Deviation

Table 5.3.3. Mean salbutamol excretion in urine post-inhalation from MDIs, expressed as % of Nominal Dose.

Treatment Method (n = 13)	**USAL0.5**		**USAL24†**		**USAL24Pre**		**USAL24Post**		**USALMET**	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Ventolin*	2.9	0.9	50.3	7.9	29.2	9.1	47.5	8.3	18.3	7.8
Airomir	3.6	1.6	42.1	14.1	21.1	7.0	38.6	13.5	17.5	9.1
Salamol	3.4	1.6	47.2	9.7	21.4	5.6	43.8	9.2	22.4	6.5
Part 2 Study (with charcoal blockade)										
Ventolin*	2.6	1.2	14.9	2.7	7.2	2.7	12.3	2.4	5.1	2.5
Airomir	3.3	1.9	20.0	6.4	10.4	3.9	16.7	5.8	6.2	3.7
Salamol	3.6	1.7	25.1	6.5	13.6	5.8	21.5	6.8	7.9	4.5

* Ventolin Evohaler; † TRD = USAL24; SD = Standard Deviation

Table 5.3.4. Mean salbutamol excretion in urine post-inhalation from MDIs, expressed as % of estimated Delivered Dose.

Treatment Method (n = 13)	USAL0.5		USAL24†		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Ventolin*	3.6	1.2	62.7	9.4	36.3	11.0	59.1	10.0	22.8	9.7
Airomir	4.5	2.1	54.1	18.3	27.0	8.9	49.5	17.6	22.5	11.9
Salamol	4.4	2.2	60.9	13.0	27.6	7.1	56.5	12.1	28.9	8.6
Part 2 Study (with charcoal blockade)										
Ventolin*	3.3	1.5	18.7	3.4	9.0	3.4	15.4	3.1	6.4	3.2
Airomir	4.2	2.4	25.4	8.3	13.2	5.0	21.2	7.7	8.0	4.8
Salamol	4.6	2.1	31.8	8.4	17.3	7.6	27.2	8.6	9.9	5.6

* Ventolin Evohaler; † TRD = USAL24; SD = Standard Deviation

Table 5.3.5. Mean salbutamol excreted in urine post-inhalation from MDIs, expressed as % of Recovered Dose.

Treatment Method (n = 13)	USAL0.5		USAL24†		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Ventolin*	5.9	2.4	-	-	57.7	14.6	94.1	2.4	36.3	14.4
Airomir	9.4	5.5	-	-	51.6	11.2	90.6	5.5	39.0	13.8
Salamol	7.1	3.1	-	-	45.4	9.4	92.9	3.1	47.5	8.7
Part 2 Study (with charcoal blockade)										
Ventolin*	17.7	7.1	-	-	48.7	16.0	82.3	7.1	33.7	14.6
Airomir	17.3	8.7	-	-	52.4	10.2	82.7	8.7	30.3	11.4
Salamol	15.8	11.3	-	-	53.2	16.0	84.2	11.3	31.0	14.3

* Ventolin Evohaler; † TRD = USAL24; SD = Standard Deviation

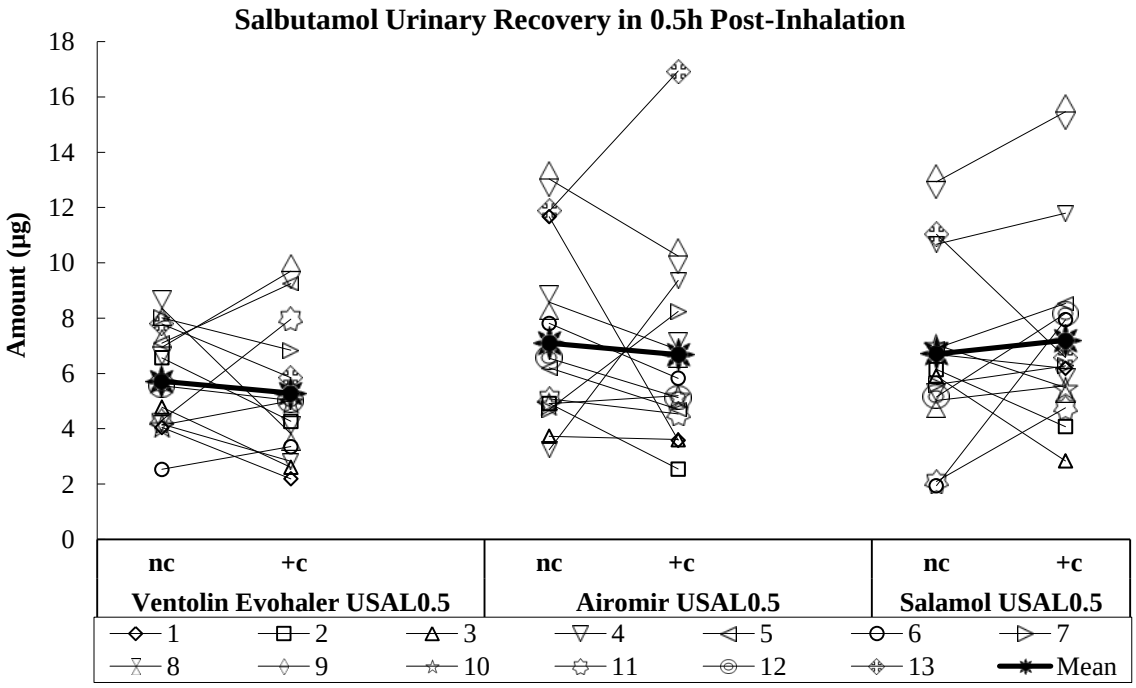


Figure 5.3.1. Comparative salbutamol urinary excretion at 0.5h post-inhalation without and with charcoal ingestion.

Numerals represent individual volunteers. nc = no charcoal ingestion; +c = with charcoal ingestion.

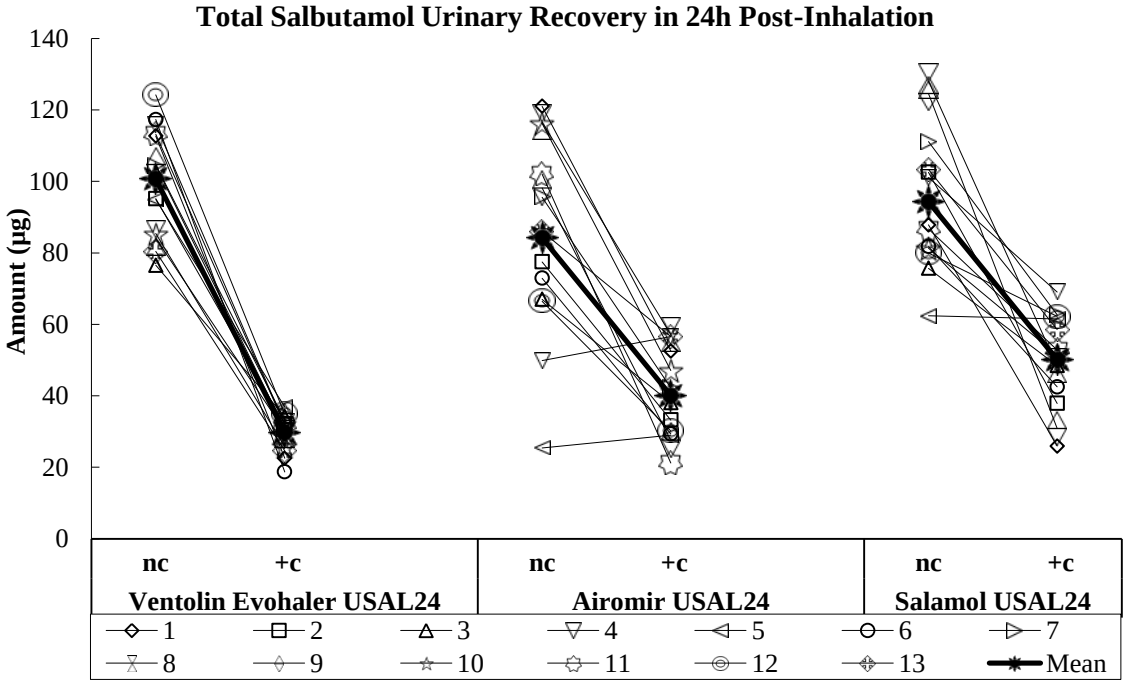


Figure 5.3.2. Comparative total salbutamol urinary excretion during 24h post-inhalation without and with charcoal ingestion.

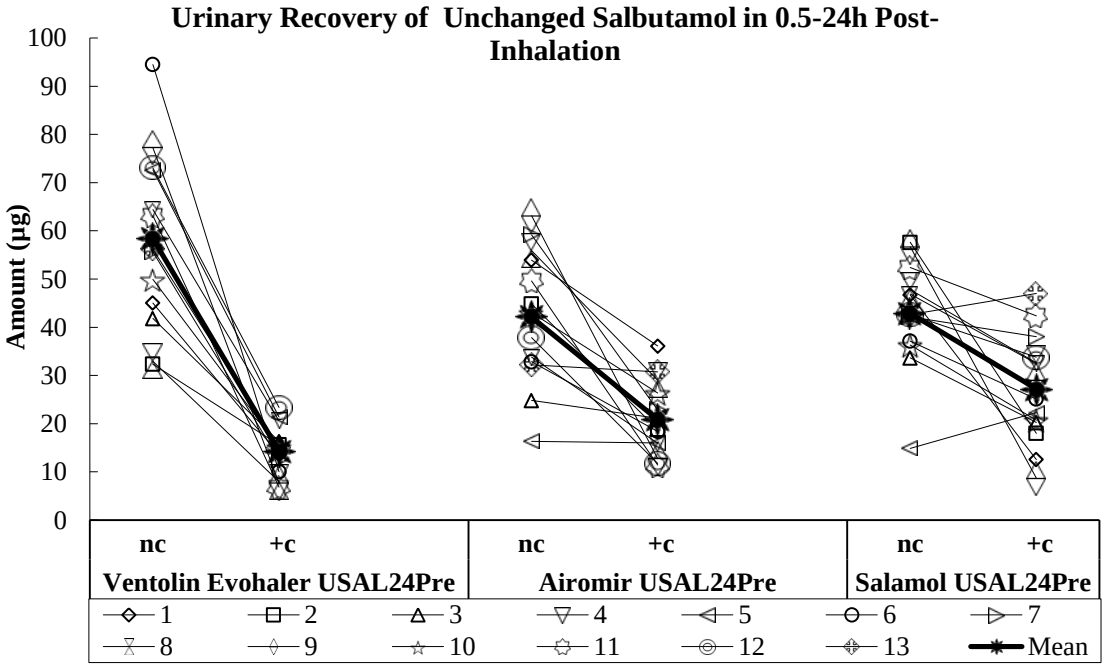


Figure 5.3.3. Comparative unchanged salbutamol urinary excretion during 0.5-24h post-inhalation without and with charcoal ingestion.

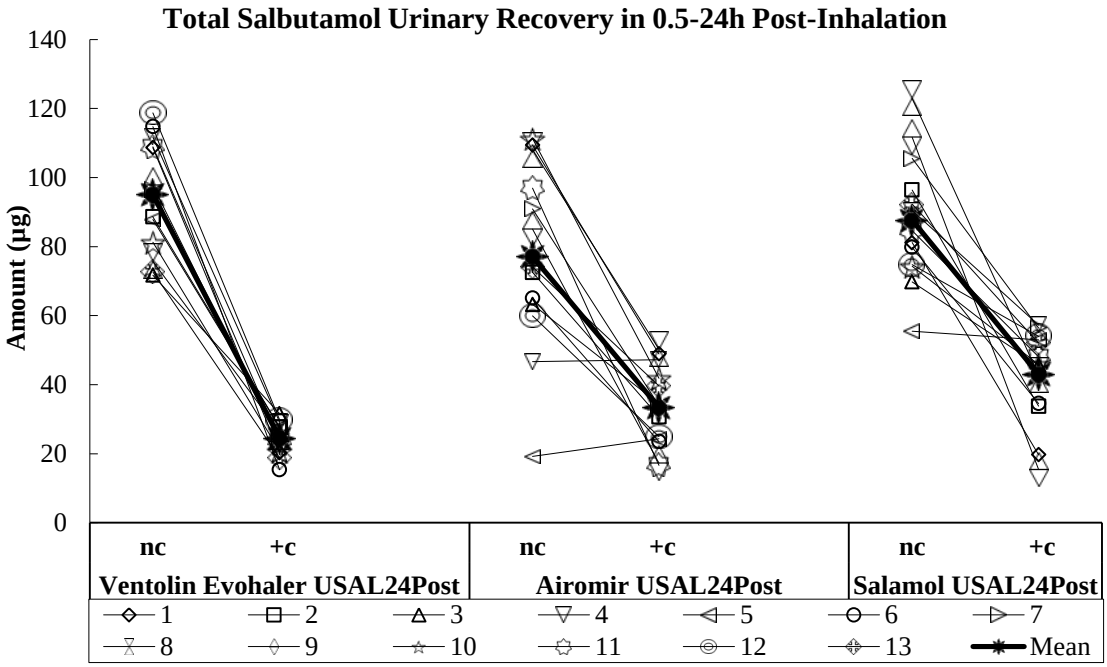


Figure 5.3.4. Comparative total salbutamol urinary excretion during 0.5-24h post-inhalation without and with charcoal ingestion.

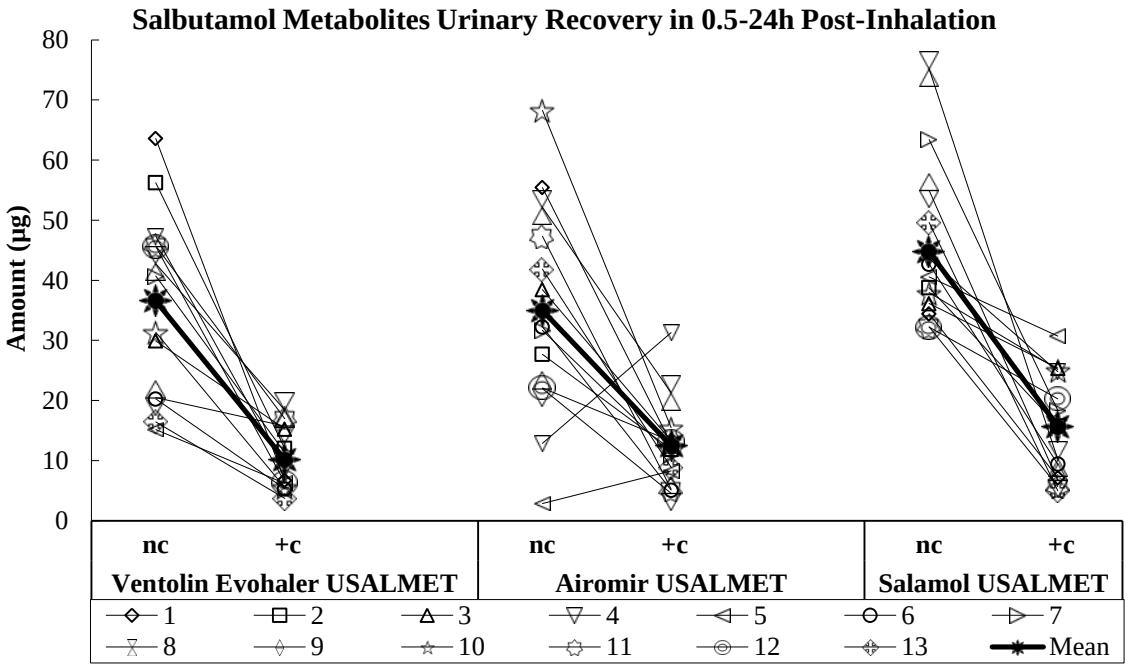


Figure 5.3.5. Comparative salbutamol metabolites urinary excretion during 0.5-24h post-inhalation without and with charcoal ingestion.

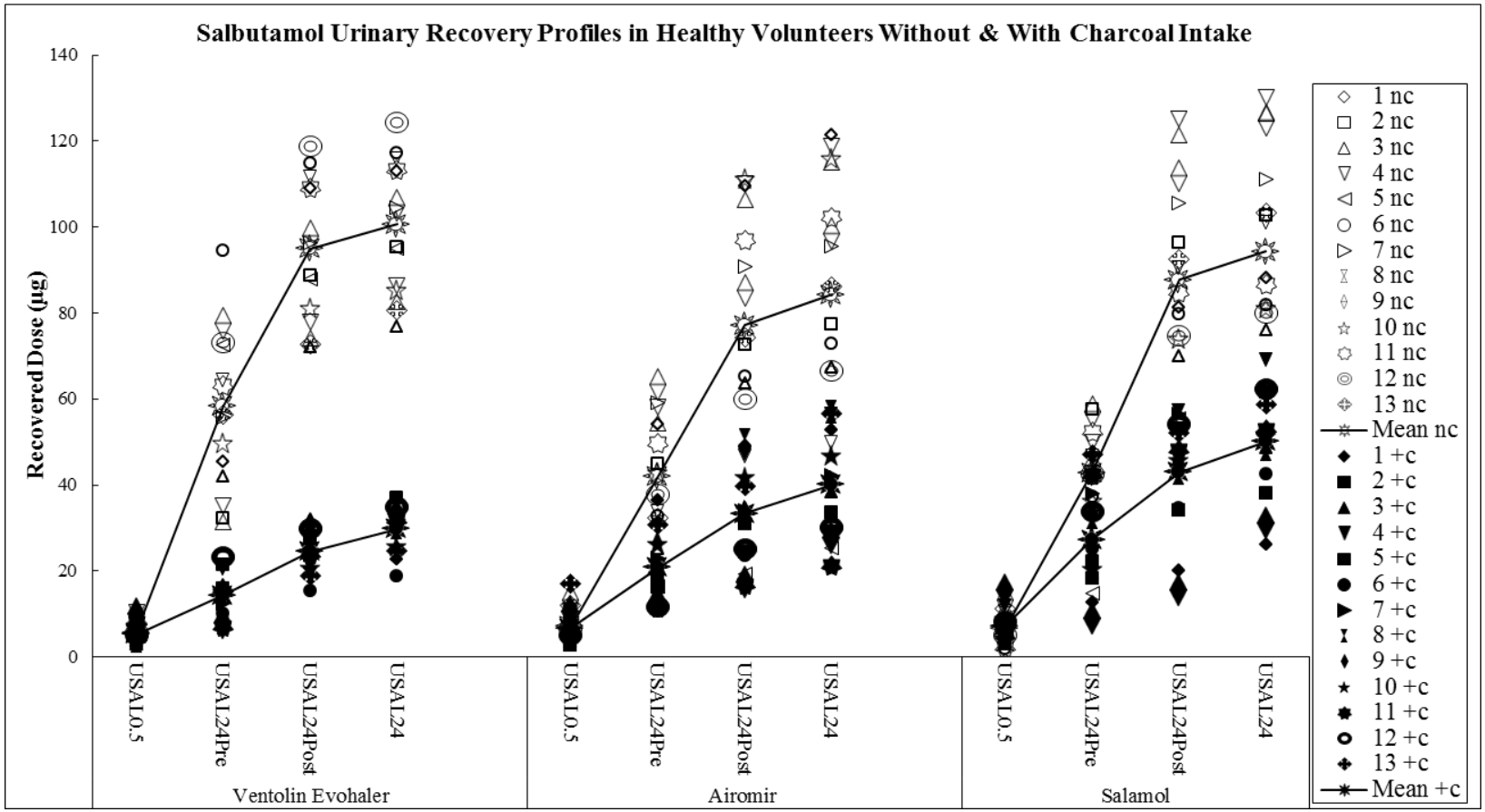


Figure 5.3.6. Comparative salbutamol urinary recovery profiles obtained post-inhalation without and with charcoal ingestion.

Numerals represent individual volunteers. nc = no charcoal ingestion; +c = with charcoal ingestion.

Table 5.3.6. *In-Vivo* Equivalence and Statistical Significance of salbutamol urinary excretion post-inhalation without charcoal ingestion.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p* value	*In-Vivo* Equivalence		Mean Difference (µg)	90% CI		*p* value	Statistical Similarity
				LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
USAL0.5 NC	Ventolin Evohaler	Airomir	0.84	0.59	1.19	0.783	No	No	-1.39	-3.47	0.70	0.438	Yes
		Salamol	0.92	0.65	1.30	1.000	No	No	-0.99	-3.07	1.09	0.884	Yes
	Airomir	Salamol	1.09	0.77	1.55	1.000	No	No	0.40	-1.68	2.48	1.000	Yes
USAL24 NC	Ventolin Evohaler	Airomir	1.27	1.00	1.60	0.091	No	No	16.48	-1.46	34.42	0.147	Yes
		Salamol	1.08	0.85	1.36	1.000	No	Yes	6.35	-11.60	24.29	1.000	Yes
	Airomir	Salamol	0.85	0.67	1.07	0.373	No	Yes	-10.13	-28.07	7.81	0.643	Yes
USAL24Pre NC	Ventolin Evohaler	Airomir	1.41	1.05	1.88	0.044	No	No	16.23	3.22	29.29	0.029	No
		Salamol	1.36	1.01	1.82	0.080	No	No	15.49	2.46	28.53	0.039	No
	Airomir	Salamol	0.97	0.72	1.29	1.000	No	Yes	-0.77	-13.80	12.27	1.000	Yes
USAL24Post NC	Ventolin Evohaler	Airomir	1.32	1.02	1.70	0.064	No	No	17.86	0.31	35.41	0.092	No
		Salamol	1.09	0.85	1.40	1.000	No	Yes	7.33	-10.22	24.88	1.000	Yes
	Airomir	Salamol	0.83	0.64	1.07	0.312	No	No	-10.53	-28.08	7.02	0.564	Yes
USALMET nc	Ventolin Evohaler	Airomir	1.17	0.74	1.87	1.000	No	No	1.61	-11.86	15.07	1.000	Yes
		Salamol	0.77	0.48	1.22	0.636	No	No	-8.16	-21.6	5.30	0.551	Yes
	Airomir	Salamol	0.65	0.41	1.04	0.154	No	No	-9.77	-23.23	3.70	0.344	Yes

[a] EMA, 2009; [b] Parameswaran, 1999; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit; NC or nc = No Charcoal

Table 5.3.7. *In-Vivo* Equivalence and Statistical Significance of salbutamol urinary excretion post-inhalation with charcoal ingestion.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p* value	*In-Vivo* Equivalence		Mean Difference (µg)	90% CI		*p* value	Statistical Similarity
				LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
USAL0.5C	Ventolin Evohaler	Airomir	0.81	0.58	1.12	0.467	No	No	-1.39	-3.73	0.96	0.582	Yes
		Salamol	0.72	0.52	1.00	0.103	No	No	-1.92	-4.26	0.43	0.231	Yes
	Airomir	Salamol	0.89	0.64	1.24	1.000	No	No	-0.53	-2.87	1.81	1.000	Yes
USAL24C	Ventolin Evohaler	Airomir	0.77	0.60	0.98	0.062	No	No	-10.27	-19.62	-0.91	0.062	No
		Salamol	0.61	0.48	0.77	0.0003	No	No	-20.38	-29.73	-11.03	0.0002	No
	Airomir	Salamol	0.79	0.62	1.00	0.107	No	No	-10.11	-19.47	-0.76	0.067	No
USAL24PreC	Ventolin Evohaler	Airomir	0.68	0.47	0.99	0.083	No	No	-6.55	-14.29	1.19	0.205	Yes
		Salamol	0.54	0.37	0.78	0.003	No	No	-12.90	-20.64	-5.15	0.003	No
	Airomir	Salamol	0.79	0.55	1.15	0.511	No	No	-6.35	-14.09	1.40	0.230	Yes
USAL24PostC	Ventolin Evohaler	Airomir	0.77	0.58	1.01	0.120	No	No	-8.88	-17.73	-0.03	0.098	No
		Salamol	0.60	0.45	0.79	0.001	No	Yes	-18.46	-27.31	-9.61	0.0003	No
	Airomir	Salamol	0.78	0.59	1.03	0.160	No	No	-9.58	-18.43	-0.731	0.067	Yes
USALMETc	Ventolin Evohaler	Airomir	0.83	0.52	1.33	1.000	No	No	-2.33	-8.41	3.75	1.000	Yes
		Salamol	0.68	0.43	1.09	0.227	No	No	-5.57	-11.65	.51	0.149	Yes
	Airomir	Salamol	0.82	0.51	1.30	1.000	No	No	-3.24	-9.32	2.84	0.723	Yes

[a] EMA, 2009; [b] Parameswaran, 1999; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit; C or c = Charcoal

The comparison of the two studies of the three MDIs without and with charcoal block is given in Table 5.3.8 (Figure 5.3.7) while Table 5.3.9 (Figure 5.3.8) shows their overall summary. The mean paired differences between the amounts of salbutamol excreted in 0.5h were statistically similar between the two parts of the same MDI study (n=13) while all other studied parameters showed significant differences (Table 5.3.8). Similar trend was observed when the three MDIs were considered together (n=39) (Table 5.3.9).

Table 5.3.8. Statistical comparison of salbutamol urinary excretion from the MDIs between Parts 1 and 2 studies.

Parameter [nc Vs (+c)]	**MDI**	**Mean paired Difference**	**95% CI**		***t value***	***p value***	**Statistical Similarity**
			LL	**UL**			
USAL0.5	Ventolin*	0.43	-0.99	1.85	0.661	0.5212	Yes
	Airomir	0.43	-1.80	2.66	0.416	0.6845	Yes
	Salamol	-0.50	-2.21	1.21	-0.639	0.5347	Yes
USAL24	Ventolin*	76.19	66.01	86.37	16.309	<0.0001	No
	Airomir	50.83	34.55	67.11	6.803	<0.0001	No
	Salamol	51.38	35.36	67.41	6.987	<0.0001	No
USAL24Pre	Ventolin*	44.05	32.70	55.40	8.458	<0.0001	No
	Airomir	21.24	12.23	30.26	5.134	0.0002	No
	Salamol	15.66	5.91	25.42	3.499	0.0044	No
USAL24Post	Ventolin*	70.47	59.71	81.24	14.261	<0.0001	No
	Airomir	43.73	28.16	59.31	6.119	0.0001	No
	Salamol	44.68	29.27	60.09	6.319	<0.0001	No
USALMET	Ventolin*	26.42	17.17	35.68	6.222	<0.0001	No
	Airomir	22.49	10.62	34.36	4.128	0.0014	No
	Salamol	29.02	18.57	39.47	6.052	0.0001	No

* Ventolin Evohaler

Table 5.3.9. Statistical comparison of salbutamol urinary excretion from the MDIs between Parts 1 and 2 studies.

Parameter [nc Vs (+c)	**Mean paired Difference**	**95% CI**		***t value***	***p value***	**Statistical Similarity**
		LL	**UL**			
USAL0.5	0.12	-0.84	1.08	0.249	0.8047	Yes
USAL24	53.08	44.53	61.63	12.572	<0.0001	No
USAL24Pre	26.99	20.35	33.62	8.236	<0.0001	No
USAL24Post	52.96	44.56	61.37	12.752	<0.0001	No
USALMET	25.98	20.38	31.57	9.403	<0.0001	No

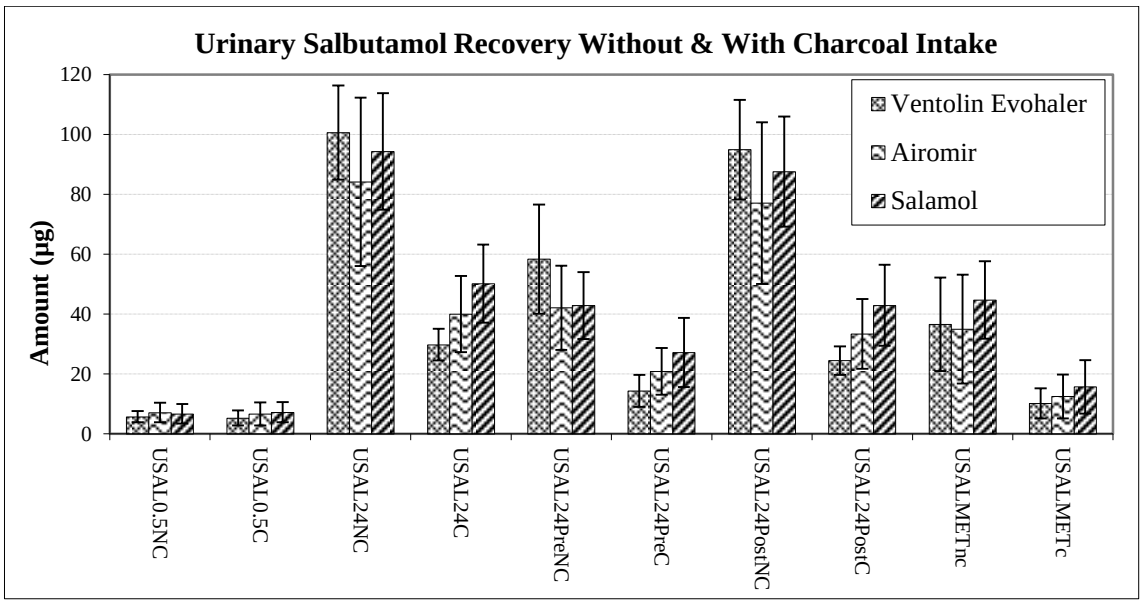


Figure 5.3.7. Comparison of salbutamol urinary excretion from the MDIs between Parts 1 and 2 studies.

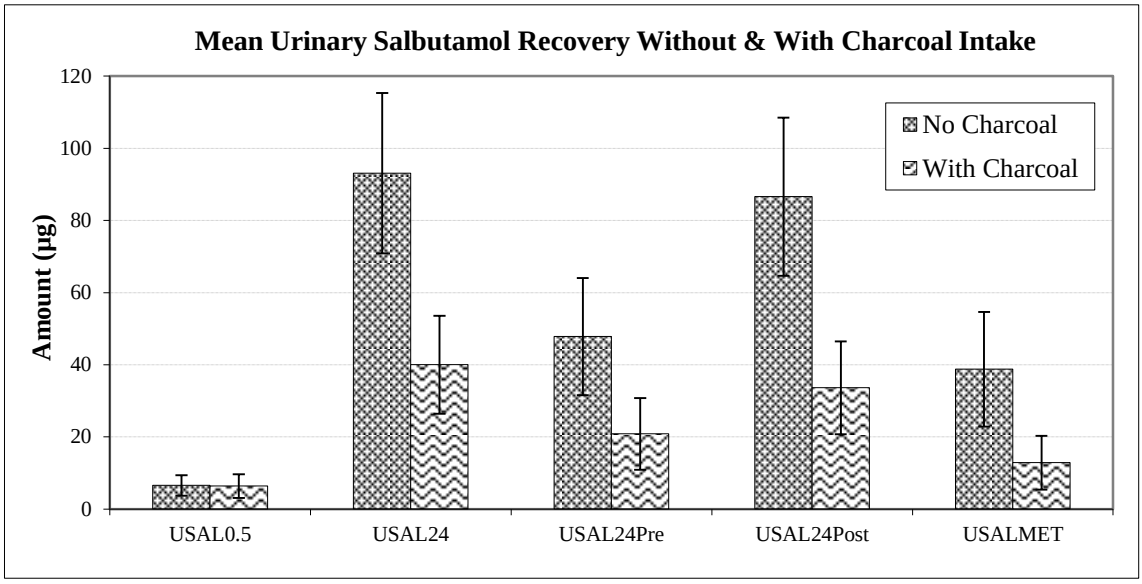


Figure 5.3.8. Comparison of salbutamol urinary excretion from the MDIs between Parts 1 and 2 studies.

Table 5.3.10 shows trends of *in-vitro* and *in-vivo* correlation between MDI performance metrics and salbutamol urinary excretion post-inhalation.

Table 5.3.10. *In-vitro* and *in-vivo* correlation trends between MDI performance metrics and salbutamol urinary excretion.

Parameter	Ventolin* µg	Airomir µg	Salamol µg	Trend (in decreasing order)
FPD	78.4	91.4	89.4	Airo ≥ Sala > Evo
%FPF (%TED)	44.4	52.4	49.6	Airo ≥ Sala > Evo
S0toF	87.9	104.7	100.0	Airo ≥ Sala > Evo
USAL0.5 nc	5.7	7.1	6.7	Airo ≥ Sala > Evo
USAL0.5 c	5.3	6.7	7.2	Sala ≥ Airo > Evo
IP+CPM	98.2	82.9	90.6	Evo > Sala > Airo
IP	88.8	69.7	80.1	Evo > Sala > Airo
USAL24Pre nc	58.4	42.1	42.9	Evo > Sala > Airo
USAL24Pre c	14.3	20.9	27.2	Sala > Airo > Evo
USAL24Post nc	95.0	77.1	87.6	Evo > Sala > Airo
USAL24Post c	24.5	33.4	43.0	Sala > Airo > Evo
TED (ACI)	176.6	174.3	180.0	Sala > Evo > Airo
Amount left in device (ACI)	41.2	40.6	48.9	Sala > Evo > Airo
Amount left in device (nc)	39.4	43.8	44.7	Sala > Airo > Evo
Amount left in device (c)	40.8	42.1	41.2	Airo > Sala > Evo
Mean amount left in device	40.5	42.2	44.9	Sala > Airo > Evo
USAL24 nc	100.7	84.2	94.4	Evo > Sala > Airo
USAL24 c	24.5	33.4	43.0	Sala > Airo > Evo

* Ventolin Evohaler

5.3.5 Discussion: *In-Vivo* Equivalence of Salbutamol HFA MDIs

The results of Part 1 Study (Table 5.3.2 & Table 5.3.6) suggest that the amount of salbutamol excreted in urine in 0.5h post-inhalation (USAL0.5NC) with Ventolin Evohaler, Airomir and Salamol do not meet EMA (2009) *in-vivo* equivalence criteria despite being statistically similar. Since urinary excretion in the first 0.5h post-inhalation is believed to be derived from the inhaled dose deposited in the lungs (Hindle and Chrystyn, 1992), these findings suggest that the three MDIs differ in their relative lung bioavailability. This may have clinical consequences in differing efficacies as lung deposition is related to improved spirometry (Chrystyn et al., 1998; Tomlinson et al., 1999; Mazhar et al., 2008; Fahimi et al., 2011).

Total systemic bioavailability measured as salbutamol urinary excretion over 24h post-inhalation (USAL24) again suggests that the three MDIs were not *in-vivo* equivalent

although this was statistically similar amongst them (Table 5.3.2 & Table 5.3.6). This finding suggests that these MDIs may have different safety profiles.

These findings support MHRA directive to prescribe MDIs with their brand names (Chrystyn and Price, 2009) and are in line with FDA's view of therapeutic inequivalence of salbutamol MDIs (Ventolin HFA, Proventil HFA and ProAir HFA) (FDA Orange Book online).

The excretion of free salbutamol over 0.5-24h post-inhalation was statistically similar only between Airo Vs Sala (Table 5.3.2 & Table 5.3.6). However, sulphate conjugated salbutamol (USALMETnc) excreted during this period was statistically similar amongst the three MDIs, which thereby resulted in their similar (p>0.05) total salbutamol (USAL24PostNC). However, none of them was *in-vivo* equivalent amongst them with respect to these parameters.

The USAL0.5 results expressed as % of the nominal dose of this study for Ventolin Evohaler (HFA) (Table 5.3.3) are comparable to those reported by others for Ventolin CFC MDI (Hindle and Chrystyn, 1992 & 1994; Hindle et al., 1995 & 1997; Chege et al., 1998; Silkstone et al., 2002a; Tomlinson et al., 2003). The USAL0.5 values (normalised for single puff) obtained in this study for Airomir are similar to those reported by Clark and Lipworth (1996b). However, these investigators used 12 puffs of Airomir and did not determine total systemic bioavailability (USAL24). USAL24 mean values reported in this thesis for Ventolin Evohaler are lower than those reported by Hindle and Chrystyn (1992, 1994), Hindle et al. (1997) and Silkstone et al. (2002a) but slightly higher than those of Chege et al. (1998). The apparent differences could be due to subject cohort and the sample size. The similarities of USAL0.5 amongst these studies indicate that urinary excretion of salbutamol in the first 0.5h post-inhalation may be independent of formulation and adult subject variables. Also, studies with other inhaled drugs further indicate that 0.5h urinary excretion post-inhalation is not influenced by the type of inhaled drug. This has been shown with urinary PK studies on inhaled sodium cromoglycate (Aswania et al., 1999; Aswania and Chrystyn, 2001 & 2002), nedocromil (Aswania et al., 1998), gentamicin (Nasr and Chrystyn, 1997; Al-Amoud et al., 2005), formeterol (Nadarassan et al., 2007), terbutaline (Abdelrahim et al., 2011) and beclometasone (Said et al., 2012). Hence, this index of relative bioavailability to the lungs is found to be robust and can discriminate between various formulations of the same inhaled drugs and/or different drugs.

Regulatory agencies recommend using charcoal blockade to exclude GI absorption of the swallowed portion of inhaled drugs to assess their pulmonary deposition to determine bioequivalence of inhalers (EMA, 2009). Hence, to explore lung deposition, oral absorption was prevented by coadministration of oral activated charcoal. Researchers have shown that charcoal could prevent 92–98% of oral absorption of salbutamol (Ward et al., 2000) which is similar to that reported for terbutaline (Borgstrom and Nilsson, 1990). The effectiveness of charcoal blockade of GI absorption has been confirmed by other investigators for salbutamol (Silkstone et al., 2000 & 2002b, Moore et al., 2017). Further, this technique has also been applied to inhaled nedocromil (Aswania et al., 1998), sodium cromoglycate (Aswania et al., 1999; Aswania and Chrystyn, 2001 & 2002), terbutaline (Abdelrahim et al., 2011) and beclometasone dipropionate (Said et al., 2012). Therefore, salbutamol excreted in urine post-inhalation with activated charcoal would have entered systemic circulation after absorption from the lungs.

In Part 2 Study with charcoal block, USAL0.5c was again statistically similar amongst the three MDIs but not *in-vivo* equivalent (Table 5.3.2 & Table 5.3.7). These results are also statistically similar to those of Part 1 Study where no charcoal blockage was used (t-test, Table 5.3.8 & Table 5.3.9). The statistical similarity and the mean differences found between two legs of the current study for USAL0.5 amounts also highlight the reproducibility of urinary PK method. Statistically similar 0.5h amounts were also reported for Ventolin CFC (Tomlinson, 2000; Silkstone et al., 2000 & 2002a; Tomlinson et al., 2003). Moore et al. (2017) have also reported similar $AUC_{0–0.5h}$ plasma profiles for Evo MDI, Diskus DPI and a Unit Dose development DPI when administered without and with charcoal. These findings reaffirm that both plasma levels and urinary excretion of inhaled salbutamol in the first 0.5h are indicative of pulmonary absorption only. Hence, it is highly likely that systemic exposure due to oral absorption of the swallowed portion of inhaled salbutamol occurs after the first half hour. This is consistent with previous observations that GI absorption of inhaled short-acting β_2-agonists is negligible as compared to the overall systemic absorption from the inhaled dose in the first 0.5h post-inhalation (Girodet and Molimard, 2005).

Further, significant statistical differences in urinary excretion of salbutamol were observed for USAL24, USAL24Pre, USAL24Post and USALMET between Parts 1 and 2 of the studies, both within the same MDI (n=13; Table 5.3.8) and when the three MDIs were taken together (n=39; Table 5.3.9). These findings concur with other studies

(Silkstone et al., 2000 & 2002b; Ward et al., 2000, Abdelrahim et al., 2011; Said et al., 2012; Moore et al., 2017).

The results (Table 5.3.5) indicate that lung exposure achieved from the first 0.5h after inhalation of salbutamol without charcoal (USAL0.5NC) from Ventolin Evohaler, Airomir and Salamol represented 6%, 9% and 7%, respectively, of the total exposure (USAL24NC). The corresponding lung exposure achieved with charcoal blockage (USAL0.5C) for these MDIs respectively represented 18%, 17% and 16% of the total exposure (USAL24C). It should be noted that the absorption, distribution and elimination of salbutamol through the lungs is ongoing and not complete within the first 0.5h post-inhalation. This is evident from continued salbutamol urinary recoveries over 24h in the presence of Charcoal blockade. This is further reflected in the trend where increases in USAL0.5C amounts were indicative of corresponding increases in USAL24C amounts of these MDIs confirming that with the charcoal blockade, systemic exposure was mainly contributed by the dose delivered to the lungs (Table 5.3.2). Hence, the net systemic exposure [(USAL0.5C / USAL24C) – (USAL0.5NC / USAL24NC)] that may have been derived from the lung deposition is respectively 12%, 8% and 9% for these MDIs. This is reflected in the trend seen with USAL24NC amounts (Evo > Sala > Airo) (Table 5.3.10).

A trend is observed between USAL0.5C amounts and USAL24C, USAL24PreC, USAL24PostC and USALMETc of the three MDIs where the differences noted in the former parameter was translated into similar rank order of latter parameters (Table 5.3.2). This trend is also consistent when assessed as % of USAL24C where these parameters respectively constituted ~17%, ~50%, ~83% and ~31% of the total systemic exposure (Table 5.3.5). These findings suggest that charcoal block of the swallowed salbutamol was effective and that systemic salbutamol mainly originated from lung deposition. These findings further highlight that urinary excretion of salbutamol in the first 0.5h can reliably predict the overall systemic exposure.

The mean (SD; µg) total systemic exposure (USAL24) was reduced by 70.90 (17.0), 44.16 (27.3) and 44.18 (25.5) (representing 30%, 48% and 53%) for Evo, Airo and Sala, respectively with charcoal block. These differences in systemic exposure are more likely due to differences in the swallowed portion of the inhaled salbutamol (Section 5.2.4.2). These MDIs have different formulation excipients and plume characteristics. The *in-vitro* deposition (µg) in IP of ACI (resembling throat) was 88.8, 69.9 and 80.1,

respectively, for these MDIs (representing 50%, 40% and 45% of TED), and highlights the likelihood and contribution of varying oropharyngeal deposition in human subjects. Hence, differences in the magnitude of charcoal block reflect on the relative variation in the swallowed proportion of the dose from these MDIs. Interestingly, this magnitude of charcoal block is of the same order of decreasing *in-vitro* IP deposition (Evo > Sala > Airo).

Further, the magnitude of charcoal block for Evo in this study is similar to that reported by Moore et al. (2017) measured via plasma salbutamol.

In Part 1 Study, the ratios of USAL24PreNC / USALMETnc for Evo, Airo and Sala were 1.60, 1.20 and 0.96, respectively. In Part 2 Study with the co-administration of activated charcoal, their respective ratios of USAL24PreC / USALMETc were 1.41, 1.67 and 1.73. These ratios reflect on their respective USAL0.5C amounts in the increasing rank order of Evo > Airo > Sala.

In Part 1 Study, the ratios of the proportion of total unchanged salbutamol to its metabolites [(USAL0.5NC + USAL24PreNC) / USALMETnc] were 1.75, 1.41 and 1.11, respectively, for Evo, Airo and Sala and this trend was in the decreasing rank order of Evo > Airo > Sala. In Part 2 Study, their corresponding ratios [(USAL0.5C + USAL24PreC) / USALMETc] were 1.93, 2.20 and 2.19, indicating that higher proportion of unchanged salbutamol than its metabolite was in the increasing rank order of Evo > Sala ≥ Airo. The *in-vitro* deposition in ACI beyond IP (throat) as impactor mass (S0toF) mimics the dose delivered to the respiratory tract beyond oropharynx and reaching to the lungs (Table 5.2.2). Interestingly, the results of *in-vitro* studies reveal similar trend in increasing impactor mass (S0toF) and FPD depositions Evo > Sala > Airo. This trend is reversed with IP deposition which decreased in the same rank order. This suggests that more dose may have been swallowed with Evo than the other two MDIs. This is reflected in the larger mean paired differences between Parts 1 and 2 (nc or NC Vs c or C) for USAL24 and USAL24Post amounts (Table 5.3.8). The results also indicate that GI absorption of the swallowed proportion of salbutamol was blocked by charcoal in the same decreasing rank order (Evo > Sala > Airo). Higher swallowed amounts found with Evo as compared to Airo and Sala are more likely due to the differences in their formulations with consequent differences in their plume characteristics and spray speed of the emitted dose (see Section 5.2.4.2). These findings

highlight the presence of link between the results of *in-vitro* and *in-vivo* studies (Table 5.3.10).

Higher amounts of unchanged salbutamol would suggest that more active drug is available for clinical effects since its sulphate ester metabolite (USALMET) is not pharmacologically active (Evans et al., 1973; Morgan et al., 1986; Morgan, 1990). The total unchanged salbutamol (USAL0.5 + USAL24Pre; µg) was 19.6, 27.6 and 34.4 respectively for Evo, Airo and Sala. Therefore, their relative amounts would predict their relative efficacy (Tomlinson et al., 2003). Based on relative recoveries of unchanged salbutamol and assuming all other contributing variables constant, the results of Part 2 Study with charcoal blockage suggest that the efficacy of these MDIs would be in the increasing rank order of Evo > Airo > Sala.

Ward et al. (2000) have shown that ~60% of (unchanged) salbutamol is absorbed into systemic circulation from the swallowed fraction of the inhaled dose from Ventolin CFC MDI. The results in this thesis for Evo reveal that this (USAL24Pre) was ~29% of the nominal dose in Part 1 (without charcoal) and ~7% in Part 2 (with charcoal) (Table 5.3.3). However, the amount recovered as USAL24PreNC was 4 times more than that of USAL24PreC and therefore suggests that ~75% of USAL24PreNC may have originated from the swallowed portion of the inhaled dose. USAL24PreNC and USAL24PreC for Airo were ~21% & ~10% and for Sala were ~21% & ~14% of the nominal dose, respectively. The GI absorption of salbutamol (USAL24PreNC) correlates well with the IP depositions found with *in-vitro* studies with these MDIs (Table 5.2.2) which were in decreasing rank order of Evo > Sala > Airo.

The total systemic exposure may have role in systemic effects (Fowler et al., 2001; Lipworth et al., 2005; Moore et al., 2017). The differences in systemic absorption of unchanged salbutamol (USAL24Pre) from these MDIs may result in differences in systemic exposure with consequent differences in systemic effects and safety profiles. The results in this thesis suggest that USAL24PreNC amounts of the three MDIs were not *in-vivo* equivalent (Table 5.3.6). Whether this continued GI absorption of swallowed unchanged salbutamol has any role in continued relief from bronchodilation is not clear.

The USAL24PreNC and USALMETnc were respectively 29% and 18% of the nominal Evo dose, and total excretion (USAL24NC) was 50% of the dose (Table 5.3.3). The corresponding values for Ventolin CFC reported by Hindle and Chrystyn (1992) were

24%, 34% and 57%. The results reported by Silkstone et al. (2002b & 2002a) were 27-30% and 28% of the nominal Ventolin CFC dose respectively, and total excretion was 57-60% of the dose. Although USAL24PreNC compares well with theirs, USALMETnc and USAL24NC reported in this thesis are lower than those reported by them. These differences are highly likely due to sample cohort having variation in individual drug metabolism. Nevertheless, Chege et al. (1998) have reported total excretion of 47.6% of Ventolin CFC dose which is similar to that found in the current study.

The USAL24PreNC and USALMETnc for Airo were 21% and 18% of the nominal Airo dose respectively, and USAL24NC was 42% of the dose. These values for Sala were 21%, 22% and 47%. These three parameters were statistically similar between these two MDIs (Table 5.3.6). These findings also reflect on their similar FPD, S0toF, %FPF and TED found in *in-vitro* studies (Table 5.2.7). On the other hand, their USAL24PreNC was significantly different from that of Evo while USALMETnc and USAL24NC were similar amongst them. This is reflected in their statistical differences Vs Evo for FPD, S0toF and %FPF, and similarity of TED amongst them. The differences and similarities amongst these three MDIs have their origins in their formulation which influenced their dose delivery characteristics (see Section 5.2.4.2).

5.3.5.1 *In-vitro in-vivo* correlation trends

In-vitro equivalence studies can potentially serve as surrogates for *in-vivo* bioequivalence studies (de Matas et al., 2008). Aerodynamic particle size distributions (APSD) using ACI broadly indicate likely deposition behaviour of inhaled products in the respiratory tract. Using APSD profile, it is possible to correlate *in-vitro* performance of an MDI to the pharmacokinetic profile of its active ingredient(s) in human subjects.

Lung deposition of salbutamol is related to its efficacy and therapeutic benefit and total systemic bioavailability to safety (Hindle and Chrystyn, 1992). The amount of salbutamol recovered in urine in the first 0.5h (USAL0.5) post-inhalation is directly related to the amount of salbutamol delivered to the lungs (Silkstone et al., 2002c).

Silkstone et al. (2002c) and Srichana et al. (2005) have reported linear relationships between pharmacokinetic indices of lung bioavailability of salbutamol and the respirable doses (FPD) delivered by nebulizer and dry powder inhaler (DPI), respectively. Further, de Matas et al. (2008) reported a clear link between FPD and relative lung bioavailability and efficacy of salbutamol sulphate dry powder inhalers.

The results in this thesis for salbutamol HFA MDIs also show a link between FPD and relative bioavailability to the lungs (USAL0.5) (Table 5.3.10). Larger FPD was related to larger USAL0.5 as evident from similar trend of *in-vitro* and *in-vivo* performance parameters of the three MDIs (Airo ≥ Sala > Evo).

Total delivered dose (TDD) to human subjects could not be determined. Since the amount of salbutamol left in MDI devices is similar to those found with devices used for APSD studies using ACI, it is assumed that TDD would be similar to TED determined during particle size analyses (Table 5.3.10). Hence, TED would mimic the total systemic availability (USAL24) of inhaled salbutamol from these MDIs and would include systemic availability from both major pathways of systemic absorption, i.e., via respiratory and gastrointestinal tracts. The TED reflects similar trend (Sala > Evo > Airo) to that of USAL24NC (Evo ≥ Sala > Airo) given that the mean differences between Sala and Evo are very small and that these two parameters are statistically similar amongst the three MDIs (Table 5.3.2 & Table 5.3.6).

In-vitro APSD studies have shown that IP (throat) deposition for these three MDIs ranged from 50% to 40% of TED (Evo > Sala > Airo). This would suggest that the oropharyngeal deposition of the dispensed dose of salbutamol would have marked contribution in its total systemic availability (USAL24). Hence, greater USAL24NC was observed with Evo as compared to the other two MDIs (Evo > Sala > Airo). This trend was reversed for Evo with charcoal block study (Sala > Airo > Evo) indicating that its larger swallowed proportion was prevented from GIT absorption. This similar reversing trend has also been observed between USAL24PreNC Vs USAL24PreC and USAL24PostNC Vs USAL24PostC for the three MDIs highlighting the significant oropharyngeal deposition.

Borgström et al. (2006) have shown that variability of *in-vivo* lung deposition of inhaled drug is related to the variability in its throat deposition. Hence, a higher deposition in the throat would result in a lower lung deposition and vice versa. The results of current study support their findings. The *in-vitro* ratios of FPD/IP, FPM/IP and S0toF/IP (Table 5.2.6) were all in the same order of *in-vivo* USAL0.5, a surrogate for lung deposition (Airo ≥ Sala > Evo) (Table 5.3.10).

The limitation of relating *in-vitro* APSD to *in-vivo* lung deposition patterns are well known (Mitchell and Nagel, 2003; Dunbar and Mitchell, 2005; Mitchell et al., 2007b;

Newman and Chan, 2008). The induction port does not mimic the oropharyngeal region of the respiratory tract while the use of a constant airflow rate at a fixed period of time is not representative of a human subject's inhalation flow profile. The temperature and humidity inside ACI are unlike those inside the lungs (Labiris and Dolovich, 2003). Prediction of *in-vivo* outcomes in individual subjects from *in-vitro* measurements is further complicated by demographic and physiological factors, such as inter-individual differences in breathing pattern, airways calibre, inter-subject variability in oropharyngeal deposition due to different airway geometries and mouth openings, high humidity conditions in the lungs, airways clearance mechanisms or inhalation technique amongst other variables (Labiris and Dolovich, 2003; de Matas et al., 2008; Daley-Yates et al., 2014; Cheng et al., 2015). The mechanisms of particle deposition in the respiratory tract, therefore, are difficult to comprehensively simulate. Despite all these limitations, the current work has broadly identified links between *in-vitro* and *in-vivo* studies.

5.3.6 Conclusions: Salbutamol HFA MDIs *In-Vivo* Study

The results of current work suggest that Ventolin Evohaler, Airomir and Salamol were not *in-vivo* equivalent to each other based on their differences in relative lung and systemic bioavailability assessed using urinary PK in healthy subjects. Hence, caution should be exercised if a change of salbutamol HFA MDI brand is desired.

The charcoal blockade suggested that urinary excretion of inhaled salbutamol in the first 0.5h was related to the lung deposition and was independent of MDI brand. These findings confirm that systemic exposure due to oral absorption of the swallowed portion of inhaled salbutamol occurs after the first half hour.

In-vitro studies showed a broad correlation to *in-vivo* studies. Trends in FPD and TED were reflected in similar trends in relative lung and systemic bioavailability.

6 Chapter 6: *In-Vitro* and *In-Vivo* Equivalence of Ventolin Evohaler Without and With Spacers

6.1 Overview

Inhaling a dose from an MDI requires coordination between actuation and inhalation. However, many patients face difficulty in correctly inhaling the dose from MDIs (Chrystyn and Price, 2009; Laube et al. 2011; Lavorini, 2013 & 2014; Lavorini et al., 2014). To overcome this problem, MDIs are used with spacers (Newman, 2004; Lavorini and Fontana, 2009; Nikander et al., 2014). Nevertheless, different spacers can induce change in the aerosol plume characteristics of a drug, which are spacer and drug specific, and vary between spacers, and with different formulations of the same drug for the same spacer (Ahrens et al., 1995; Barry and O'Callaghan, 1996, 1997; Lipworth and Clark, 1998, Dubus et al., 2001, McCabe et al., 2012; Johnson et al., 2016). Accordingly, APSD studies on spacers attached to Ventolin Evohaler were carried out to assess their comparative *in-vitro* performance when used alone and with spacers. This *in-vitro* work was followed-up by *in-vivo* studies using urinary pharmacokinetic method (Hindle and Chrystyn, 1992).

This chapter is organised into separate sections comprising of *in-vitro* and *in-vivo* equivalence studies on Ventolin Evohaler without and with spacers.

6.2 *In-Vitro* Equivalence of Ventolin Evohaler Without and With Spacers-Aerodynamic Particle size Characterisation

The objectives of this study are to:

a) determine APSD of Ventolin Evohaler without and with spacers using ACI,
b) investigate *in-vitro* equivalence between MDI alone and with spacer, and
c) identify *in-vitro* equivalence between different spacers attached to Ventolin Evohaler.

The *in-vitro* drug delivery characteristics of MDI without and with spacer were assessed with respect to their Critical Quality Attributes (CQAs) (ICH Q8, 2009). FPD is a key performance indicator and provides a common ground to compare inhaler treatment methods (Section 3.3.4). However, APSD profiles were also assessed as stage groups (EMA, 2006 & 2009).

6.2.1 Materials and Methods

6.2.1.1 Materials and Equipments

See Chapter 3 (Section 3.3.1.1).

6.2.1.2 Test MDI

Ventolin Evohaler™ (Evo).

6.2.1.3 Test Spacers

Volumatic™ (VOL), AeroChamber Plus™ (AERO) and Able™ (ABLE) spacer (Figure 3.3.1).

6.2.2 Study Design

APSD studies using ACI were performed as per Protocols 3.3.1 and 3.3.2 (Sections 3.3.2.3 & 3.3.2.4) in compliance to pharmacopoeial requirements (BP, 2005; USP28-NF23, 2005; Ph. Eur., 2011) (see Chapter 3 Methodology). Briefly, one puff of primed Ventolin Evohaler was discharged into ACI operated at a flow rate of 28.3 L/min for 8.5 seconds to allow 4L of air to pass through it. The second puff was similarly discharged after 30 seconds. This procedure was repeated with a randomly selected spacer. All spacers were pre-treated with lukewarm soapy water to disseminate electrostatic charge and drip dried after a water rinse (Section 2.4). The amounts of

salbutamol deposited in the MDI device, spacer and ACI components and stages were recovered and quantified using a validated HPLC method (see Chapter 4).

6.2.3 Deposition Profiles, CQAs and Data Analysis

The data for critical performance metrics, APSD profiles and spacer deposition were analysed as per pharmacopoeial requirements and regulatory guidelines, and described in Chapter 3 (Section 3.3.3).

6.2.4 APSD and ACI Stage Grouping

See Chapter 3 (Section 3.3.4).

6.2.5 Statistical Analysis

See Chapter 3 (Section 3.3.5).

6.2.6 Results: *In-Vitro* Equivalence of Ventolin Evohaler Without and With Spacers

The mean amount (n=5) of two actuations of salbutamol deposited on various components and stages of ACI recovered from Ventolin Evohaler alone and with VOL, AERO and ABLE spacers are shown in Table 6.2.1. Data on their respective individual runs is given in Appendices 5.2.3.1 and 6.2.6.1 to 6.2.6.3. Figure 6.2.1 and Figure 6.2.2 respectively show their complete mean APSD and cumulative particle size deposition profiles. Their comparative CQAs and stage group depositions are shown in Figure 6.2.3 and Figure 6.2.4 and summaries provided in Table 6.2.2 to Table 6.2.6. Dose delivery efficiency of these treatment methods is compared in Table 6.2.7. Table 6.2.8 to Table 6.2.12 present their *in-vitro* equivalence and statistical comparisons. The characteristics of these spacers are given in Table 6.2.13 and Figure 6.2.5 displays dimensional measurements.

Mass balance and TED of Ventolin Evohaler, Evo+VOL, Evo+AERO and Evo+ABLE were respectively within 5% and 25% of labelled claim of 100 µg metered dose (Table 6.2.1). This confirms system suitability of the ACI equipment used. Hence, the results generated are valid and accurate (Christopher et al., 2003).

Table 6.2.1. APSD of Ventolin Evohaler alone and with spacers.

Identity	Ventolin Evohaler			Evo+VOL			Evo+AERO			Evo+ABLE			Notations
	µg	SD	RSD	µg	SD	RSD	µg	SD	RSD	µg	SD	RSD	
MDI Canister Valve	5.0	0.5	9.9	6.2	0.9	14.4	5.8	1.2	21.5	6.7	2.0	29.6	a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 μg per puff
MDI Actuator	36.2	2.9	8.1	23.1	1.3	5.8	24.1	1.9	7.8	20.0	2.7	13.5	
Spacer	-	-	-	74.9	6.1	8.2	90.6	6.7	7.4	87.6	4.4	5.0	
ACI IP (Throat)	88.8	7.4	8.3	5.5	1.9	34.4	5.0	1.1	22.5	5.1	0.9	18.1	b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).
ACI S-0	2.1	0.2	8.8	1.9	0.5	24.4	2.3	0.7	30.2	2.3	0.4	17.0	
ACI S-1	2.8	0.3	12.5	3.8	0.7	18.0	4.4	0.8	17.4	4.5	0.5	11.8	
ACI S-2	4.6	0.6	12.1	5.5	1.3	23.4	6.1	0.9	14.5	6.3	0.9	14.1	
ACI S-3	18.4	2.2	12.1	20.1	3.5	17.2	18.9	2.4	12.9	19.9	3.4	17.0	
ACI S-4	35.2	2.6	7.3	33.2	1.9	5.7	28.7	2.3	8.2	27.9	4.5	16.3	
ACI S-5	19.9	1.9	9.7	19.0	1.6	8.7	15.4	1.4	8.8	17.3	2.1	12.4	c = TED (Total Emitted Dose Ex-Actuator); Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve and Actuator (mouth piece).
ACI S-6	3.4	0.3	8.3	4.0	0.9	23.0	2.7	0.7	25.4	5.0	1.1	21.3	
ACI S-7	0.6	0.1	13.2	0.9	0.1	14.4	0.9	0.3	33.0	1.9	0.3	16.2	
ACI Filter	0.9	0.2	19.9	0.9	0.2	21.0	1.0	0.2	17.6	2.0	0.3	13.5	
Total Recovery (μg)	217.8	8.9	4.1	199.1	5.0	2.5	205.8	6.0	2.9	206.6	2.1	1.0	
% Recovery[a]	108.9	4.4	4.1	99.5	2.5	2.5	102.9	3.0	2.9	103.3	1.0	1.0	
Mass Balance[b] (μg)	212.8	8.6	4.0	192.9	4.4	2.3	200.1	6.3	3.1	199.9	2.1	1.1	d = TDD (Total Delivered Dose Ex-Spacer); Recovery calculated with respect to Nominal Dose (ND) and excludes deposition on Canister Valve, Actuator (mouth piece) and spacer.
% Recovery	106.4	4.3	4.0	96.5	2.2	2.3	100.0	3.1	3.1	99.9	1.1	1.1	
TED[c] (μg)	176.6	7.6	4.3	169.8	4.5	2.7	176.0	4.5	2.5	179.8	1.1	0.6	
% TED	88.3	3.8	4.3	84.9	2.3	2.7	88.0	2.2	2.5	89.9	0.6	0.6	
TDD[d] (μg)	-	-	-	94.9	3.5	3.7	85.3	4.7	5.6	92.2	3.7	4.0	
% TDD	-	-	-	47.4	1.8	3.7	42.7	2.4	5.6	46.1	1.9	4.0	

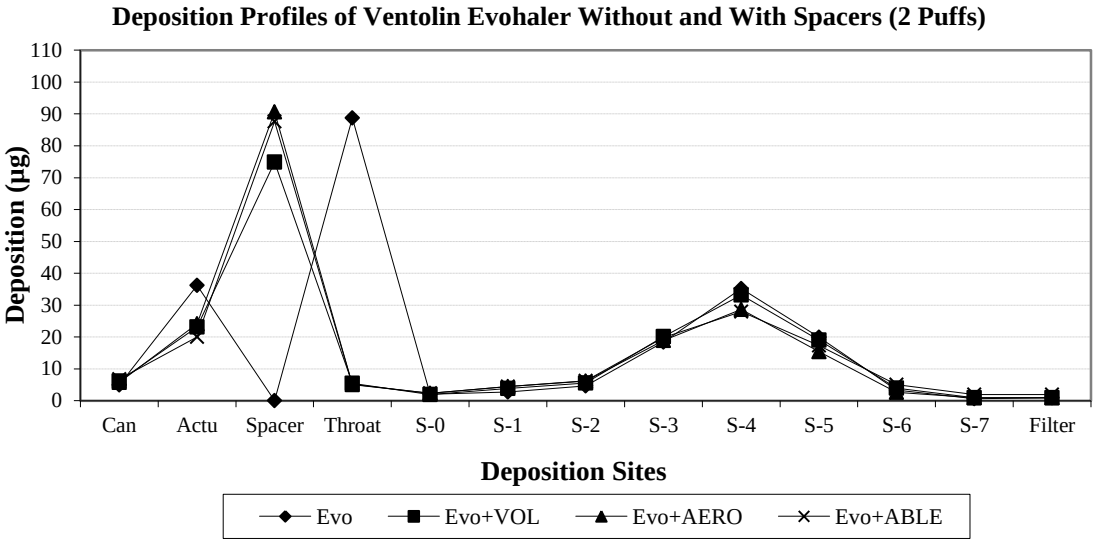


Figure 6.2.1. Mean APSD profiles of Ventolin Evohaler alone and with spacers.

Can = MDI Canister; Actu = MDI Actuator

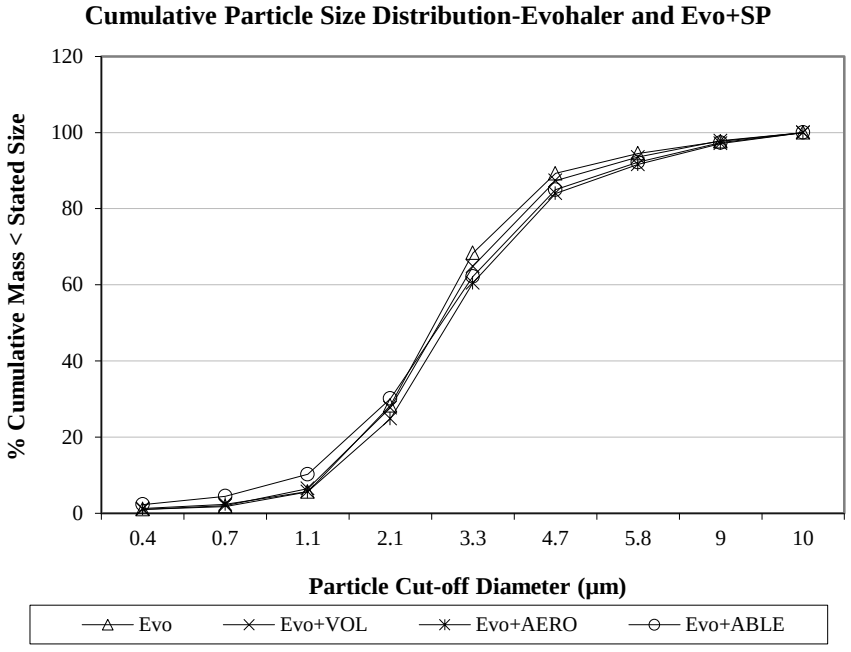


Figure 6.2.2. Mean percent cumulative particle size deposition profiles of Ventolin Evohaler alone and with spacers.

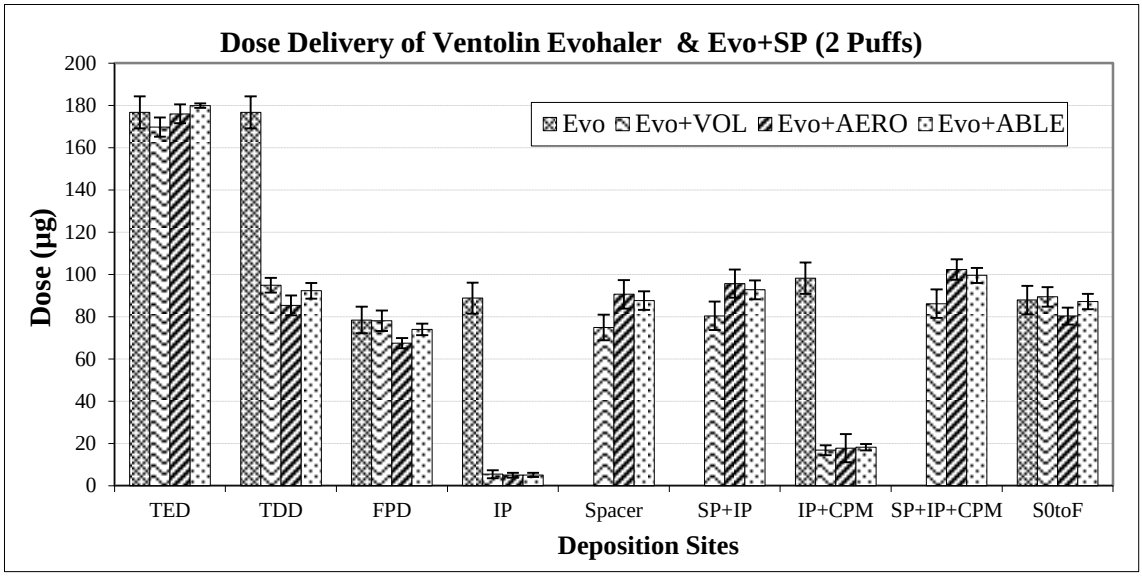


Figure 6.2.3. Dose delivery characteristics of Ventolin Evohaler alone and with spacers.

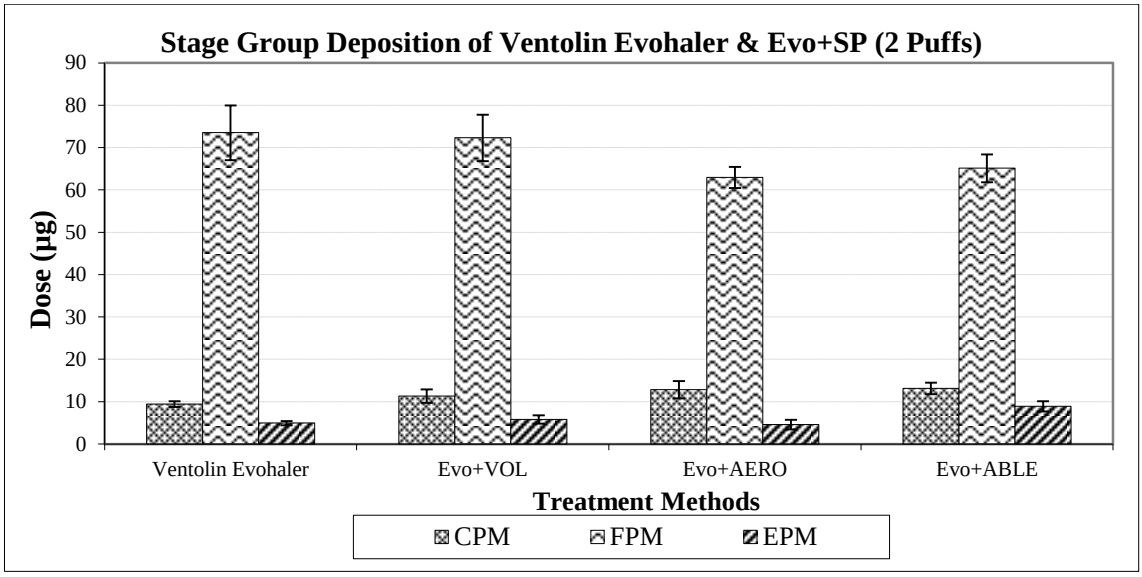


Figure 6.2.4. Stage group deposition of Ventolin Evohaler alone and with spacers.

TED (ex-actuator) of Evo alone compared to three spacers (Evo+SP) was *in-vitro* equivalent and statistically similar (Table 6.2.2 & Table 6.2.8). However, TDD (ex-spacer) was not *in-vitro* equivalent between them and also showed significant statistical differences. TDD of Evo (=TED) was about twice the TDD of Evo+SP.

TED (ex-actuator) of Evo+SP was *in-vitro* equivalent between them. However, statistically similarity was not observed between Evo+VOL Vs Evo+ABLE (Table 6.2.2 & Table 6.2.9). On the other hand, *in-vitro* equivalent TDD between spacer treatment methods was observed only for Evo+VOL Vs Evo+ABLE and Evo+AERO Vs Evo+ABLE while TDD of the three Evo+SP was statistically similar.

IP deposition of Evo alone Vs Evo+SP showed *in-vitro* inequivalence and significant statistical difference (Table 6.2.2 & Table 6.2.11; Figure 6.2.3). This is because about 50% of TED of Evo deposited in IP. Interestingly, while being *in-vitro* inequivalent, IP deposition of Evo alone was statistically similar to the combined deposition of SP+IP with spacer treatment methods.

In contrast, statistical difference of SP+IP deposition between Evo+VOL Vs Evo+AERO was significant and Vs Evo+ABLE was marginally significant with the least salbutamol SP+IP deposition in Evo+VOL; nonetheless, both were *in-vitro* inequivalent (Table 6.2.2 & Table 6.2.12). Similar trend was observed with dose retained in these two spacers, however, the statistical differences were significant. Nevertheless, SP+IP and SP depositions of Evo+AERO Vs Evo+ABLE were both statistically similar and *in-vitro* equivalent. IP depositions of the three Evo+SP were statistically similar between them while being *in-vitro* inequivalent.

Deposition of IP+CPM (non-respirable fraction) from Evo alone was 5 to 6 times more than that of Evo+SP (Table 6.2.2; Figure 6.2.3). However, only Evo+VOL showed lower ratio of SP+IP+CPM deposition (non-respirable fraction) than IP+CPM deposition of Evo alone while the other two spacers attached to Evo had higher non-respirable fraction. This trend was also observed when IP+CPM and SP+IP+CPM were assessed as %TED. IP+CPM depositions assessed as % TDD were ~3 times more with Evo alone than those from Evo+SP; Evo+VOL showed the lowest deposition while the other two spacers followed the above noted trend.

Table 6.2.2. Dose delivery and deposition in ACI of Ventolin Evohaler alone and with spacers.

Treatment Method	TED		TDD		SP		IP		SP+IP		IP+CPM		SP+IP+CPM		S0toF	
	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD
Ventolin*	176.63	7.55	176.63	7.55	-	-	88.78	7.36	-	-	98.21	7.40	-	-	87.85	6.77
Evo+VOL	169.78	4.55	94.89	3.50	74.89	6.13	5.51	1.90	80.40	6.75	16.81	2.31	91.70	6.29	89.38	4.63
Evo+AERO	175.96	4.47	85.33	4.75	90.62	6.73	5.02	1.13	95.64	6.67	17.86	2.46	108.48	4.84	80.32	4.01
Evo+ABLE	179.84	1.10	92.25	3.73	87.59	4.40	5.13	0.93	92.72	4.44	18.23	1.48	105.82	3.52	87.12	3.66

* Ventolin Evohaler; SD = Standard Deviation

Table 6.2.3. Dose delivery and deposition in ACI as %TED and %TDD of Ventolin Evohaler alone and with spacers.

Treatment Method	TDD_ED		SP_ED		IP_ED		SP+IP_ED		IP+CPM_ED		SP+IP+CPM_ED		S0toF_ED		IP_DD		IP+CPM_DD		S0toF_DD	
	%ED	SD	%ED	SD	%ED	SD	%ED	SD	%ED	SD	%ED	SD	%ED	SD	%DD	SD	%DD	SD	%DD	SD
Ventolin*	100.00	-	-	-	50.25	3.48	-	-	55.59	3.31	-	-	49.75	3.48	50.25	3.48	55.59	3.31	49.75	3.48
Evo+VOL	55.93	2.73	44.07	2.73	3.25	1.12	47.32	3.16	9.92	1.51	53.99	2.94	52.68	3.16	5.83	2.09	17.76	2.74	94.17	2.09
Evo+AERO	48.53	3.02	51.47	3.02	2.85	0.60	54.32	2.78	10.16	1.46	61.63	1.58	45.68	2.78	5.85	1.10	20.85	1.77	94.15	1.10
Evo+ABLE	51.30	2.25	59.84	2.03	2.85	0.51	51.55	2.23	10.14	0.83	58.84	1.69	48.45	2.23	5.56	1.01	19.75	1.12	94.44	1.01

ED = TED; DD = TDD

Table 6.2.4. FPD, Stage groups, MMAD and GSD of Ventolin Evohaler alone and with spacers.

Treatment Method	FPD		FPM		EPM		CPM		MMAD		GSD	
	µg	SD	µg	SD	µg	SD	µg	SD	um	SD		SD
Ventolin*	78.42	6.28	73.49	6.46	4.93	0.51	9.43	0.68	2.68	0.03	1.56	0.02
Evo+VOL	78.08	4.80	72.28	5.50	5.80	1.00	11.30	1.60	2.77	0.07	1.67	0.06
Evo+AERO	67.48	2.39	62.91	2.49	4.56	1.12	12.84	2.03	2.91	0.10	1.68	0.05
Evo+ABLE	74.02	2.73	65.11	3.29	8.90	1.21	13.10	1.35	2.79	0.10	1.67	0.03

Impactor mass (S0toF) deposition was both statistically similar and *in-vitro* equivalent between Evo alone Vs Evo+VOL and Vs Evo+ABLE. With Evo+AERO, slightly less S0toF deposition was noted, and comparison to Evo alone was *in-vitro* inequivalent albeit being statistically similar (Table 6.2.8). Also, S0toF of Evo+VOL Vs Evo+AERO and Evo+AERO Vs Evo+ABLE were *in-vitro* inequivalent despite these being statistically similar (Table 6.2.9).

In-vitro equivalent and statistically similar FPD was observed for Evo Vs Evo+VOL and Vs Evo+ABLE (Table 6.2.4 & Table 6.2.8). However, FPD was not *in-vitro* equivalent and had statistically significant difference between Evo Vs Evo+AERO. Amongst Evo+SP comparisons (Table 6.2.9), FPD of Evo+VOL Vs Evo+ABLE was both *in-vitro* equivalent and statistically similar. Nevertheless, FPD of Evo+VOL Vs Evo+AERO and Evo+AERO Vs Evo+ABLE were *in-vitro* inequivalent, though it was statistically similar for the latter pair.

FPD as %TED (%FPF ex-actuator) was statistically similar and *in-vitro* equivalent between Evo Vs Evo+VOL only (Table 6.2.5 & Table 6.2.8). This parameter was also statistically similar between Evo and Evo+ABLE. Amongst Evo+SP, %FPF (ex-actuator) was statistically similar only between Evo+AERO Vs Evo+ABLE and none of them were *in-vitro* equivalent (Table 6.2.5 & Table 6.2.9).

FPF as %TED (ex-actuator) of Evo alone Vs FPF as %TDD (ex-spacer) of Evo+SP was significantly different and *in-vitro* inequivalent (Table 6.2.5 & Table 6.2.8). However, FPD as %TDD (ex-spacer) was *in-vitro* equivalent and statistically similar amongst the three spacers (Table 6.2.6 & Table 6.2.9).

MMADs and GSDs of Evo alone and Evo+SP were all *in-vitro* equivalent (Table 6.2.4 & Table 6.2.10). However, there were statistically significant differences between Evo Vs Evo+AERO for MMAD, and Evo Vs the three Evo+SP for GSD.

FPM deposition was both statistically similar and *in-vitro* equivalent between Evo Vs Evo+VOL while Evo Vs Evo+ABLE it was statistically similar only (Table 6.2.4 & Table 6.2.11). Also, between Evo+SP comparisons, Evo+AERO Vs Evo+ABLE had both statistically similar and *in-vitro* equivalent FPM which was however only statistically similar between Evo+VOL Vs Evo+ABLE (Table 6.2.4 & Table 6.2.12).

CPM deposition was not *in-vitro* equivalent either between Evo Vs Evo+SP or amongst the spacer treatment methods (Table 6.2.4, Table 6.2.11 & Table 6.2.12). Nevertheless, CPM of Evo Vs Evo+VOL and Evo Vs Evo+AERO, and the three Evo+SP treatment methods was statistically similar.

EPM deposition was *in-vitro* inequivalent between Evo Vs Evo+SP and amongst the three Evo+SP treatments (Table 6.2.4, Table 6.2.11 & Table 6.2.12). However, EPM was statistically similar between Evo Vs Evo+VOL and Evo Vs Evo+AERO, and Evo+VOL Vs Evo+AERO.

Summary of Results

TED (ex-actuator), FPD (%S0toF), MMAD and GSD were *in-vitro* equivalent with all treatment methods, i.e., Evo alone Vs Evo+SP.

Comparisons between Ventolin Evohaler alone and with attached VOL were *in-vitro* equivalent with respect to TED (ex-actuator), FPD, FPF (%TED), MMAD, GSD, S0toF and FPM deposition.

Comparisons between Evo alone and when attached to AERO were *in-vitro* equivalent with respect to TED (ex-actuator), MMAD and GSD.

Comparisons between Evo alone and with attached ABLE were *in-vitro* equivalent with respect to TED (ex-actuator), FPD, MMAD, GSD and S0toF.

Moreover, TED (ex-actuator), FPF (%TDD), MMAD and GSD were *in-vitro* equivalent amongst Evo+SP treatment methods. Also, TDD (ex-spacer) of Evo+VOL Vs Evo+ABLE and Evo+AERO Vs Evo+ABLE were *in-vitro* equivalent.

TED, TDD, FPD, S0toF, MMAD and GSD were *in-vitro* equivalent for Evo+VOL Vs Evo+ABLE.

TED, MMAD and GSD were *in-vitro* equivalent for Evo+VOL Vs Evo+AERO. TDD, S0toF and FPD were each ~5 µg lower with Evo+AERO.

TED, TDD, MMAD and GSD were *in-vitro* equivalent between Evo+AERO and Evo+ABLE. S0toF and FPD were statistically similar between them but were not *in-vitro* equivalent. S0toF and FPD were both ~3 µg lower for Evo+AERO.

Table 6.2.5. FPD and stage groups as %TED of Ventolin Evohaler alone and with spacers.

Treatment Method	FPF (%)		FPM_ED		EPM_ED		CPM_ED	
	%TED	SD	%TED	SD	%TED	SD	%TED	SD
Ventolin*	44.41	3.31	41.62	3.49	2.79	0.26	5.34	0.28
Evo+VOL	46.01	2.94	42.60	3.36	3.41	0.56	6.67	1.06
Evo+AERO	38.37	1.58	35.76	1.40	2.60	0.67	7.32	1.27
Evo+ABLE	41.16	1.69	36.21	1.95	4.95	0.67	7.29	0.77

* Ventolin Evohaler; SD = Standard Deviation; ED = TED; DD = TDD

Table 6.2.6. FPD and stage groups as %TDD of Ventolin Evohaler alone and with spacers.

Treatment Method	FPF (%)		FPM_DD		EPM_DD		CPM_DD	
	%TDD	SD	%TDD	SD	%TDD	SD	%TDD	SD
Ventolin*	44.41	3.31	41.62	3.49	2.79	0.26	5.34	0.28
Evo+VOL	82.24	2.74	76.12	3.72	6.13	1.18	11.92	1.76
Evo+AERO	79.15	1.77	73.80	2.39	5.35	1.24	15.00	1.83
Evo+ABLE	80.25	1.12	70.58	1.67	9.68	1.47	14.19	1.13

Table 6.2.7. FPD and S0toF delivery efficiency of Ventolin Evohaler alone and with spacers.

Treatment Method	FPD / SP		FPD / IP		FPD / SP+IP		FPD / IP+CPM		FPD / SP+IP+CPM		S0toF / IP		S0toF / SP+IP	
	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD
Ventolin*	-	-	0.89	0.12	-	-	0.80	0.10	-	-	1.00	0.13	-	-
Evo+VOL	1.05	0.12	17.17	11.02	0.98	0.12	4.75	0.94	0.86	0.10	19.61	12.42	1.12	0.14
Evo+AERO	0.75	0.07	13.91	2.59	0.71	0.06	3.82	0.42	0.62	0.04	16.56	3.15	0.84	0.09
Evo+ABLE	0.85	0.07	14.80	2.51	0.80	0.07	4.08	0.29	0.70	0.05	17.43	2.99	0.94	0.08

Table 6.2.8. *In-Vitro* Equivalence and Statistical Significance of CQAs of Ventolin Evohaler alone and with spacers.

Parameter	Multiple Comparisons	Mean Ratio	90% CI		*p* value	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
	Ventolin Evohaler Vs		LL	UL				LL	UL		
TED (Ex-Actuator)	Evo + VOL	1.04	0.99	1.09	0.285	Yes	3.43	-1.30	8.15	0.267	Yes
	Evo + AERO	1.00	0.96	1.05	1.000	Yes	0.34	-4.39	5.07	1.000	Yes
	Evo + ABLE	0.98	0.93	1.03	1.000	Yes	-1.60	-6.33	3.12	1.000	Yes
TDD (into ACI Throat)	Evo + VOL	1.86	1.73	2.01	<0.0001	No	40.87	35.98	45.76	<0.0001	No
	Evo + AERO	2.07	1.92	2.23	<0.0001	No	45.65	40.76	50.54	<0.0001	No
	Evo + ABLE	1.91	1.78	2.06	<0.0001	No	42.19	37.30	47.08	<0.0001	No
FPD	Evo + VOL	1.00	0.91	1.11	1.000	Yes	0.17	-3.97	4.31	1.000	Yes
	Evo + AERO	1.16	1.05	1.28	0.006	No	5.47	1.33	9.61	0.006	No
	Evo + ABLE	1.06	0.96	1.17	0.900	Yes	2.20	-1.94	6.34	0.773	Yes
%FPF (%TED)	Evo + VOL	0.96	0.87	1.06	1.000	Yes	-1.60	-6.34	3.15	1.000	Yes
	Evo + AERO	1.16	1.05	1.28	0.007	No	6.05	1.30	10.80	0.009	No
	Evo + ABLE	1.08	0.98	1.19	0.367	No	3.25	-1.50	8.00	0.337	Yes
%FPF (%TDD)	Evo + VOL	0.54	0.50	0.58	<0.0001	No	-37.83	-42.38	-33.28	<0.0001	No
	Evo + AERO	0.56	0.52	0.60	<0.0001	No	-34.73	-39.28	-30.18	<0.0001	No
	Evo + ABLE	0.55	0.51	0.59	<0.0001	No	-35.84	-40.39	-31.29	<0.0001	No
S0toF (S0 to S7 & Filter)	Evo + VOL	0.98	0.89	1.08	1.000	Yes	-0.76	-5.44	3.92	1.000	Yes
	Evo + AERO	1.09	0.99	1.20	0.174	No	3.77	-0.91	8.45	0.166	Yes
	Evo + ABLE	1.01	0.91	1.11	1.000	Yes	0.37	-4.31	5.05	1.000	Yes

CQA = Critical Quality Attribute; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 6.2.9. *In-Vitro* Equivalence and Statistical Significance of CQAs of Ventolin Evohaler with spacers.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p value*	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
TED (Ex-Actuator)	Evo+VOL	Evo+AERO	0.96	0.92	1.01	0.404	Yes	-3.09	-7.82	1.64	0.402	Yes
		Evo+ABLE	0.94	0.90	0.99	0.035	Yes	-5.03	-9.76	-0.30	0.033	No
	Evo+AERO	Evo+ABLE	0.98	0.93	1.03	1.000	Yes	-1.94	-6.67	2.79	1.000	Yes
TDD (into ACI Throat)	Evo+VOL	Evo+AERO	1.11	1.03	1.20	0.010	No	4.78	-0.12	9.67	0.058	Yes
		Evo+ABLE	1.03	0.95	1.11	1.000	Yes	1.32	-3.57	6.21	1.000	Yes
	Evo+AERO	Evo+ABLE	0.92	0.86	1.00	0.080	Yes	-3.46	-8.35	1.44	0.297	Yes
FPD	Evo+VOL	Evo+AERO	1.16	1.05	1.28	0.007	No	5.30	1.16	9.44	0.008	No
		Evo+ABLE	1.05	0.96	1.16	1.000	Yes	2.03	-2.11	6.17	0.956	Yes
	Evo+AERO	Evo+ABLE	0.91	0.83	1.01	0.138	No	-3.27	-7.41	0.87	0.182	Yes
%FPF (%TED)	Evo+VOL	Evo+AERO	1.20	1.09	1.32	0.001	No	7.64	2.90	12.39	0.001	No
		Evo+ABLE	1.12	1.01	1.23	0.052	No	4.85	0.10	9.60	0.044	No
	Evo+AERO	Evo+ABLE	0.93	0.84	1.03	0.448	No	-2.80	-7.55	1.95	0.572	Yes
%FPF (%TDD)	Evo+VOL	Evo+AERO	1.04	0.96	1.12	1.000	Yes	3.10	-1.45	7.64	0.344	Yes
		Evo+ABLE	1.02	0.95	1.10	1.000	Yes	1.99	-2.56	6.54	1.000	Yes
	Evo+AERO	Evo+ABLE	0.99	0.92	1.06	1.000	Yes	-1.11	-5.65	3.44	1.000	Yes
S0toF (S0 to S7 & Filter)	Evo+VOL	Evo+AERO	1.11	1.01	1.23	0.062	No	4.53	-0.15	9.21	0.061	Yes
		Evo+ABLE	1.03	0.93	1.13	1.000	Yes	1.13	-3.55	5.81	1.000	Yes
	Evo+AERO	Evo+ABLE	0.92	0.84	1.02	0.247	No	-3.40	-8.08	1.28	0.264	Yes

CQA = Critical Quality Attribute; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 6.2.10. *In-Vitro* Equivalence and Statistical Significance of MMAD and GSD of Ventolin Evohaler alone and with spacers.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p* value	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
MMAD (µm)	Ventolin Evohaler	Evo+VOL	0.97	0.92	1.02	0.652	Yes	-0.08	-0.24	0.07	0.713	Yes
		Evo+AERO	0.92	0.88	0.97	0.002	Yes	-0.23	-0.38	-0.07	0.002	No
		Evo+ABLE	0.96	0.92	1.01	0.330	Yes	-0.10	-0.26	0.05	0.360	Yes
	Evo+VOL	Evo+AERO	0.95	0.91	1.00	0.077	Yes	-0.14	-0.30	0.01	0.074	Yes
		Evo+ABLE	0.99	0.95	1.04	1.000	Yes	-0.02	-0.17	0.13	1.000	Yes
	Evo+AERO	Evo+ABLE	1.04	1.00	1.10	0.164	Yes	0.12	-0.03	0.28	0.159	Yes
GSD	Ventolin Evohaler	Evo+VOL	0.94	0.90	0.98	0.007	Yes	-0.11	-0.19	-0.02	0.010	No
		Evo+AERO	0.93	0.89	0.98	0.004	Yes	-0.11	-0.20	-0.03	0.005	No
		Evo+ABLE	0.94	0.90	0.98	0.007	Yes	-0.11	-0.19	-0.02	0.009	No
	Evo+VOL	Evo+AERO	1.00	0.95	1.04	1.000	Yes	-0.01	-0.09	0.08	1.000	Yes
		Evo+ABLE	1.00	0.96	1.04	1.000	Yes	-0.001	-0.08	0.08	1.000	Yes
	Evo+AERO	Evo+ABLE	1.00	0.96	1.05	1.000	Yes	0.01	-0.08	0.09	1.000	Yes

CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 6.2.11. *In-Vitro* Equivalence and Statistical Significance of Stage Group deposition of Ventolin Evohaler alone and with spacers.

Parameter	Multiple Comparisons	Mean Ratio	90% CI		*p* value	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
	Ventolin Evohaler Vs		LL	UL				LL	UL		
SP+IP (Spacer + Throat (MDI SP+IP = IP)	Evo + VOL	1.10	0.98	1.25	0.289	No	4.19	-1.90	10.28	0.330	Yes
	Evo + AERO	0.93	0.82	1.05	0.739	No	-3.43	-9.52	2.66	0.656	Yes
	Evo + ABLE	0.96	0.84	1.08	1.000	No	-1.97	-8.06	4.12	1.000	Yes
IP (Throat)	Evo + VOL	17.26	10.82	27.53	<0.0001	No	41.63	37.96	45.31	<0.0001	No
	Evo + AERO	17.99	11.28	28.69	<0.0001	No	41.88	38.20	45.56	<0.0001	No
	Evo + ABLE	17.49	10.97	27.90	<0.0001	No	41.82	38.15	45.50	<0.0001	No
Group 1 (CPM) Stages (S0+S1+S2)	Evo + VOL	0.84	0.68	1.04	0.271	No	0.13	-0.87	1.14	0.784	Yes
	Evo + AERO	0.74	0.60	0.92	0.011	No	-0.94	-2.36	0.49	0.395	Yes
	Evo + ABLE	0.72	0.58	0.89	0.005	No	-1.71	-3.13	-0.28	0.014	No
Group 2 (FPM) Stages (S3+S4+S5)	Evo + VOL	1.02	0.91	1.14	1.000	Yes	0.60	-3.88	5.09	1.000	Yes
	Evo + AERO	1.16	1.04	1.31	0.016	No	5.29	0.80	9.78	0.016	No
	Evo + ABLE	1.13	1.00	1.26	0.085	No	4.19	-0.30	8.68	0.076	Yes
Group 3 (EPM) Stages (S6+S7+F)	Evo + VOL	0.86	0.64	1.15	1.000	No	-0.43	-1.38	0.52	1.000	Yes
	Evo + AERO	1.10	0.82	1.47	1.000	No	0.18	-0.76	1.13	1.000	Yes
	Evo + ABLE	0.56	0.42	0.74	<0.0001	No	-1.99	-2.93	-1.04	<0.0001	No

CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 6.2.12. *In-Vitro* Equivalence and Statistical Significance of Spacer and Stage group deposition of Ventolin Evohaler with spacers.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p value*	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
SP	Evo+VOL	Evo+AERO	0.83	0.73	0.94	0.002	No	-7.86	-13.52	-2.21	0.002	No
		Evo+ABLE	0.85	0.75	0.97	0.013	No	-6.35	-12.00	-0.69	0.017	No
	Evo+AERO	Evo+ABLE	1.03	0.91	1.17	1.000	Yes	1.52	-4.14	7.17	1.000	Yes
SP+IP	Evo+VOL	Evo+AERO	0.84	0.74	0.95	0.010	No	-7.62	-13.71	-1.53	0.010	No
		Evo+ABLE	0.87	0.76	0.98	0.040	No	-6.16	-12.25	-0.07	0.047	No*
	Evo+AERO	Evo+ABLE	1.03	0.91	1.17	1.000	Yes	1.46	-4.63	7.55	1.000	Yes
IP (Throat)	Evo+VOL	Evo+AERO	1.04	0.65	1.66	1.000	No	0.24	-3.44	3.92	1.000	Yes
		Evo+ABLE	1.01	0.64	1.62	1.000	No	0.19	-3.49	3.87	1.000	Yes
	Evo+AERO	Evo+ABLE	0.97	0.61	1.55	1.000	No	-0.05	-3.73	3.63	1.000	Yes
Group 1 (CPM) Stages (S0+S1+S2)	Evo+VOL	Evo+AERO	0.88	0.71	1.09	0.825	No	0.94	-0.49	2.36	0.395	Yes
		Evo+ABLE	0.86	0.69	1.06	0.458	No	-0.77	-2.20	0.66	0.742	Yes
	Evo+AERO	Evo+ABLE	0.97	0.79	1.21	1.000	No	0.77	-0.66	2.20	0.742	Yes
Group 2 (FPM) Stages (S3+S4+S5)	Evo+VOL	Evo+AERO	1.15	1.02	1.29	0.035	No	4.69	0.20	9.17	0.038	No
		Evo+ABLE	1.11	0.99	1.24	0.176	No	3.59	-0.90	8.07	0.172	Yes
	Evo+AERO	Evo+ABLE	0.97	0.86	1.08	1.000	Yes	-1.10	-5.59	3.39	1.000	Yes
Group 3 (EPM) Stages (S6+S7+F)	Evo+VOL	Evo+AERO	1.28	0.96	1.71	0.212	No	0.62	-0.33	1.56	0.409	Yes
		Evo+ABLE	0.65	0.48	0.86	0.006	No	-1.55	-2.50	-0.61	0.001	No
	Evo+AERO	Evo+ABLE	0.50	0.38	0.67	<0.0001	No	-2.17	-3.12	-1.22	<0.0001	No

*Marginally significant; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 6.2.13. Characteristics of spacers.

Parameter	VOL	AERO	ABLE
Valve Type (Oliveira et al., 2015)	Coin	O-ring flap; Circular with internal baffle	Leaflets
Total Length with mouthpiece (external) (cm)	23.3	14.5	14.7
Mouthpiece length (external) (cm)	3.0	3.5	2.9
Length excluding mouthpiece (external)* (cm)	20.3	11.1	11.8
Length of MDI Port (cm)	1.1	1.5	1.9
Axial distance* (cm)	19.0	9.3	9.9
Total volume including mouthpiece (mL)	830	149	150
Volume of mouthpiece (mL)	10	14	7
Internal Volume excluding mouthpiece (mL)	820	135	143
Construction material	Polycarbonate	Clear copolyester[a]	transparent polypropylene[b]

[a] Asmus et al., 2003; [b] Goncalves et al., 2013
*Corrected for:
VOL: Subtracted 0.25 cm [(wall thickness 0.15 cm) + (valve protrusion 0.1 cm)]; 18.95 rounded to 19 cm
AERO: Subtracted 0.3 cm (Axial distance measured 10.8 cm at the surface of valve protrusion 0.3 cm)
ABLE: Subtracted 1.9 cm [(1.4 cm Ventolin MDI Actuator mouthpiece length) + 0.5 cm inside depth of the spacer MDI Port)]

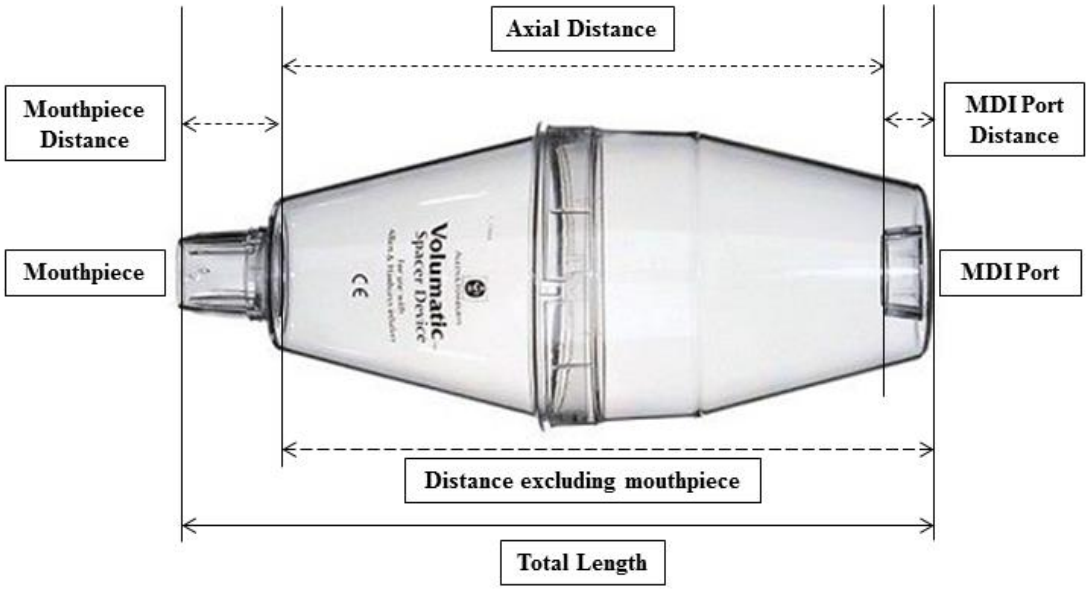


Figure 6.2.5. Spacer dimensional measurements.

6.2.7 Discussion: *In-Vitro* Equivalence of Ventolin Evohaler Without and With Spacer

EMA guideline (2009) recommends enlisting named spacer (VHC) in product summary. Spacers are used to assist patients who have either difficulty in coordinating discharge and inhaling dose from MDI such as elderly or children, or where the objective is to minimise localised and/or systemic side effect of inhaled medication. With the availability of a number of spacers, it has been necessary to determine their compatibility and suitability to be used with a given MDI medication and to challenge the claims of manufacturers. It is also prudent to investigate independently and re-affirm effectiveness of a recommended spacer in the Patient Information Leaflet (PIL) and Summary of Product Characteristics (SPC) for use with a given MDI.

In the following sections, critical performance metrics of Ventolin Evohaler (Evo) alone and with three spacers [Volumatic (VOL), AeroChamber Plus (AERO) and Able (ABLE)] have been examined separately.

6.2.7.1 TED and TDD

TDD of Evo alone was significantly greater than that of the three Evo+SP treatments. This is because about half of TED was retained within spacer. For an MDI, TED = TDD, therefore, all emitted dose is delivered. This is consistent with significantly greater deposition in IP observed with Evo alone as compared to that of Evo+SP treatment methods (Figure 6.2.3). These differences may have clinical implications (see Section 6.2.7.2).

However, TDD from Evo+SP treatment methods varied between them. Also, TDD from Evo+ABLE was slightly higher than that obtained with Evo+AERO. These differences in TDD are more likely due to differences in spacer design with consequent varying TED retained in each of them. Nevertheless, these TED (ex-actuator) and TDD (ex-spacer) (calculated by this author) are similar to those reported by Cripps et al. (2000) for Evo+VOL with respective ratios of ~1.06 and ~1.12.

6.2.7.2 Deposition in Spacer, IP and SP+IP

VOL is a diamond shaped large volume spacer. AERO is cylindrically shaped and ABLE is pear-shaped, both having relatively small volumes. Their respective reported volumes are 750 mL, 149 mL and 150 mL. The volume (including mouthpiece) of VOL

measured in this lab is 830 mL (Table 6.2.13) which differs from its often quoted volume (Mitchell et al., 1999; Cripps et al., 2000; Barry and O'Callaghan, 1996; Hall et al., 2011; Brambilla et al., 2011; Huatmann et al., 2013; Oliveira et al., 2015 & 2016). However, volumes (including mouthpiece) of AERO and ABLE measured in this lab confer to reported volumes. The respective volumes of these spacers without mouthpiece are 820 mL, 135 mL and 143 mL. The internal effective axial lengths up to the valve (excluding MDI actuator port length) of these spacers are 19 cm, 9.3 cm and 9.9 cm, respectively (Figure 6.2.5).

The internal spacer dimensions and volume excluding mouthpiece provide the effective space to emitted cloud of aerosolised drug to evaporate and form smaller particles. Plume geometry, spray velocity and impaction force of emitted dose influence deposition in spacer (Barry and O'Calaghan, 1997; Gabrio et al., 1999; McCabe et al., 2012). Ventolin Evohaler has a fast and forceful spray and therefore travels longer distance and may require more space for emitted droplets to evaporate (Barry and O'Calaghan, 1997; Gabrio et al., 1999; McCabe et al., 2012; Hautmann et al., 2013; Johnson et al., 2016). Therefore, with smaller volume and diameter of a cylindrical spacer, it is highly likely that relatively more proportion of fast moving evaporating droplets will impact on side walls and distal end of spacer and will be retained therein. This explains the likely reason of the highest spacer deposition in AERO with resultant lower TDD and *in-vitro* inequivalence between Evo+AERO and Evo+VOL.

The distance from actuator mouthpiece of MDI placed at lips to the throat of an adult human is up to 10 cm (Brambilla et al., 2011; Hautmann et al., 2013). The internal axial length (excluding spacer mouthpiece) of VOL and ABLE account for this distance while AERO is 0.7 cm short of providing for this distance (Table 6.2.13). Therefore, with AERO attached to Evo, more proportion of TED is likely to deposit in it. This is reflected in relatively larger aerosol deposition in AERO than the other two spacers; this spacer deposition decreased in order of AERO > ABLE > VOL. This reflects on their relative space and internal axial distances which increase in order of AERO > ABLE > VOL. These findings are in agreement with the work of Cripps et al. (2000) who reported higher deposition in smaller volume cylindrical Babyhaler (350 mL) than VOL when used with Evo. Hence, spacer volume and dimensions are linked to the degree of aerosol dose retention in it when used with Evo.

The discharged puff from Evo has high velocity and travels long distance (Gabrio et al., 1999; Ross and Gabrio, 1999; McCabe et al., 2012). Large volume VOL and its diamond shape therefore provide adequate space for this spray to move forward with relatively smaller impaction of spray cone on side walls and at distal end. This also provides enough time to emitted dose droplets to evaporate and form finer particles besides slowing down their velocity. This is reflected in relatively lower deposition in VOL than that was found with either AERO or ABLE; differences between them were statistically significant and *in-vitro* inequivalent (Table 6.2.12). However, the dose retained in Evo+AERO and Evo+ABLE was statistically similar and *in-vitro* equivalent. This finding is suggestive of their similar performance and is more likely due to their closely related internal volumes and axial distances.

On the other hand, TDD similarity of Evo between VOL and ABLE is more likely related to the shape of the spacer. The pear-shape of ABLE spacer may have provided enough room to the spray cone of the emitted dose and may have facilitated its forward movement alongside it by virtue of increasing cone angle of its walls with increasing distance from the MDI port and thereby may have reduced loss of drug due to impaction on walls by alignment with moving plume. This also explains the highest TDD from VOL which has wider cone angle due to its diamond shape and takes into account spray cone angle of emitted dose from Evo. These findings therefore suggest a possible link between shape, volume and diameter of spacer to dose retention and emission from them (Mazhar and Chrystyn, 2008). Other investigators have also reported similar relationship for Ventolin CFC (Mitchell et al., 1999) and sodium chromoglycate CFC (Intal) (Barry and O'Callaghan, 1995a).

IP deposition of Evo alone was about 17 times more than that of Evo+SP. Hence, IP deposition was effectively reduced by these spacers. This outcome was expected. Spacers used in this study retained a significant portion of TED (44% to 52%). This is in agreement with previously reported dose retention in VOL of Ventolin CFC (Hindle and Chrystyn, 1994, Silkstone et al., 2002a) and Evo (Cripps et al., 2000). Deposition in VOL found in this study (37 µg) is the same as reported by Cripps et al. (2000). However, their reported IP deposition is about half of that found in current study.

The dose retention of aerosol droplets and larger particles in these spacers eventually resulted in only about 3% (TED) deposition in IP. These results also show similar

deposition in IP irrespective of the type of spacer attached to Ventolin Evohaler. Interestingly, deposition in SP+IP was similar to IP deposition of Evo alone. This was also reflected when these depositions were assessed as %TED. These findings indicate that the proportion of Ventolin Evohaler delivered dose containing non-respirable particles size (> 10 um) was not affected by spacer shape and volume. Hence, generation of larger non-respirable particles is more likely related to Ventolin Evohaler device design and is intrinsic to its formulation.

The reduction in IP deposition with spacer is important clinically and from the perspective of patient compliance (Brennan et al., 2005; Lavorini, 2014). Clinically, this will reduce the amount of swallowed drug and thereby reduce systemic side effects of salbutamol. Plume temperature of Ventolin Evohaler is below zero ($^{\circ}$C) (Gabrio et al., 1999; Ross and Gabrio, 1999; McCabe et al., 2012). On actuation, the discharged puff produces "Cold Freon" effect on throat impaction (Fink, 2000; Bell and Newman, 2007). This may cause reflexive cessation of inhaling manoeuvres in some patients (Crompton, 1982; Fink, 2000; Newman, 2004) and may result in complete or partial loss of dose with clinical consequence for treatment (Melani et al., 2011). Spacers used with Evo will therefore minimise this effect.

Non-respirable fractions of Evo Vs Evo+VOL (IP+CPM Vs SP+IP+CPM) were more closely related to each other than those of the other two spacers (AERO and ABLE). This suggests that former two treatment methods produced similar non-respirable fraction of discharged dose. Non-respirable fraction of dose decreased in order of Evo+VOL > Evo MDI alone > Evo+ABLE > Evo+AERO. This trend was also observed when non-respirable fraction was assessed as %TED (IP+CPM Vs SP+IP+CPM) and %TDD (IP+CPM). The relative fractions of respirable and non-respirable dose may have implication for efficacy and safety.

6.2.7.3 Impactor Mass (S0toF)

Due to similar deposition in IP and SP+IP, statistically similar TED (TDD) entered impactor assembly (S0toF) with all treatment methods (MDI & MDI+SP). This shows that Evo alone and Evo+SP treatments of Evo were similar in delivering dose to impactor assembly. This implies that similar dose will be delivered to lower respiratory tract (HRT) whether or not a spacers is attached to it. This is perhaps surprising. However, difference between two delivery systems (MDI Vs MDI+SP) is evident from

the greater proportion of respirable dose composition of spacer treatment method. With spacer attached to Evo, about 80% of TDD consisted of FPD as compared to ~44% of Evo alone, thereby resulting in significant statistical differences and *in-vitro* inequivalence. These results further reveal that dose that reached to impactor assembly as %TDD with attached spacer was almost twice of that from MDI alone. These findings clearly highlight benefits and efficiency of spacer use (Lavorini and Fontana, 2009). Conversely, with S0toF as %TDD, statistical similarity and *in-vitro* equivalence were observed amongst all Evo+SP treatment methods. This would infer that any of these three spacers can be used with Evo. However, this inference based solely on derived data could be potentially incorrect (see Section 6.2.7.5) since it is known that differences exist between spacers (Barry and O'Callaghan, 1996, 1997; Hall et al., 2011). Besides, a given spacer can only be recommended to be used with a given MDI if the desired CQAs are similar and are found *in-vitro* equivalent in one-to-one comparison (EMA, 2006 & 2009).

6.2.7.4 Respirable Dose (FPD)

FPD obtained from Evo alone and with attached VOL was both statistically similar and *in-vitro* equivalent. This finding supports the recommendation in PIL to use VOL as an add-on device (GSK, 2018). The similarity of FPD obtained from the two delivery systems is clinically important. For clinicians, it provides smooth switch-over from Evohaler alone to EVO+VOL. If their FPD is significantly different, then this may have implications for efficacy and safety. Besides, such differences may require PK and/or PD studies to establish their comparability in EU area. Cripps et al. (2000) found slightly higher FPD (stages 2-6) with attached VOL (43 µg) than with Evo alone (34 µg) (ratio ~ 1.27). Nevertheless, these investigators concluded that two treatment methods were similar. They were of the view that this difference in FPD was unlikely to be of clinical significance due to inter-patient variability observed with inhaled drug delivery. Nonetheless, their reported FPD obtained with Evo+VOL is similar to that found in current study (41 µg) (ratio ~ 1.05) (stages 2-6).

FPD of Evo alone Vs Evo+ABLE and Evo+VOL Vs Evo+ABLE were both statistically similar and *in-vitro* equivalent. Although internal volume of ABLE is small (143 mL), however, it is pear-shaped, which may have provided enough space and span to emitted droplets of moving spray cone to evaporate and form finer particles. On the other hand, FPD of Evo+AERO was neither statistically similar nor *in-vitro* equivalent to Evo alone and Evo+VOL. Nevertheless, statistical similarity of FPD (and FPM) was observed

between AERO and ABLE while FPM was also *in-vitro* equivalent between them. Therefore, it can be concluded that similarities of FPD and FPM between AERO and ABLE are more likely due to their comparable spacer internal volumes. Taylor et al. (2003) found that when spacer shape is similar, *in-vitro* FPD of corticosteroid inhaler was not affected by increasing spacer volume from 250 mL to 1500 mL. Barry and O'Callaghan (1995a) found that increasing spacer diameter had more effect than increasing spacer volume on *in-vitro* recovery of FPD of sodium cromoglycate CFC MDI (Intal). Also, Cripps et al. (2000) reported similar FPD of Evo alone and when attached to Babyhaler. Babyhaler is a tube shaped spacer having a volume of 350 mL and length of 30 cm (Blake et al., 2012). Hence, results of ABLE spacer obtained in this project suggest that a spacer with an internal volume of 143 mL and internal effective axial length of ~10 cm with pear-shape (cone) geometry would be an optimal configuration for a spacer to be used with Evo. This finding suggests that ABLE spacer can also be used as an add-on device to Evo.

Although the FPD of Evo+AERO was neither statistically similar nor *in-vitro* equivalent to that obtained from Evo alone and Evo+VOL, yet FPD of the latter was about 6 µg more than the former (Evo+AERO). Hall et al. (2011) also found higher FPD (~8 µg) with EVO+VOL as compared to cylindrically shaped small volume Breath-a-tech spacer (218 mL). Moreover, FPD obtained from EVO+ABLE was only ~ 3 µg higher than that obtained from EVO+AERO. Even though PK and or PD studies may be required, however, clinical significance of such smaller differences probably may not be known due to difficulty of bioequivalence methods presently used to detect such small differences (García-Arieta, 2014). This shortcoming is confounded by patient factors such as underling individual variations in respiratory tract anatomy and physiology, gender, age, disease state and inhalation technique (Lipworth and Clark 1997; Chrystyn, 1999; Labiris and Dolovich, 2003; Rubin, 2010; Venegas et al., 2013).

6.2.7.5 Fine Particle Fraction

The significant differences between Fine Particle Fraction (FPF) as %TED (ex-actuator) and %TDD (ex-spacer) were obviously related to their differences in the dose that reached impactor stages (S0toF). These differences were expected. As mentioned earlier (see Section 6.2.7.1), all of emitted dose was delivered to ACI with Evo alone, and therefore TED = TDD. While with spacer attached to Evo, a large proportion of this emitted dose was retained by spacer and significantly smaller dose was delivered to ACI. This resulted in them being *in-vitro* inequivalent with respect to FPF as %TDD.

The finding that FPF as %TDD of the three spacers were all statistically similar and *in-vitro* equivalent is not surprising since these add-on devices removed most proportion of non-respirable dose from the aerosol cloud. Although these findings do suggest their similar efficiency in reducing large sized particle mass that otherwise would have deposited in IP, yet these could be misleading for comparative purposes. This is because a similar ratio of FPD to TDD could be obtained with an MDI+SP combination with a proportionally higher FPD and TDD as compared to that which has both these metrics at proportionally lower values. Also, with a disproportionate relationship between FPD and TDD, this could even show a relatively inferior recovery of either of these metrics to be equivalent or superior to comparator MDI+SP. This is because FPF is a function of two attributes, TED (or TDD) and FPD, both of which can vary independently of each other and thereby affect the resultant FPF as mentioned above. For example, Hall et al. (2011) have reported TDD and FPD of 41 µg and 37 µg, respectively, for Evo+VOL. TDD and FPD reported in this thesis are respectively 7 µg (~14%) and 2 µg (~5%) higher than they reported. This resulted in their 8.9% (ratio ~1.11) higher FPF (ex-spacer) value despite having smaller FPD. Even though the individual ratios of TDD and FPD suggest superiority of these raw data results obtained in the current study to theirs, yet their reported FPF as %TDD implies relatively better performance of VOL than was found here. This outcome assessment is therefore inherently incorrect.

On the other hand, Mitchell et al. (2009) obtained TDD and FPD of Evo+AERO of 54 µg and 49 µg, respectively, which gives ~91% as FPF. Their results for TDD and FPD are 11 µg (~20%) and 15 µg (~31%) higher than those reported in this thesis. The two results are significantly different but the disproportionate differences between TDD and FPD obtained with these two studies resulted in %FPF value which only differed by ~11.5% (ratio ~1.15), thereby suggesting similarity of the two results. Hence, inferences from %FPF regarding similarity of different treatment methods should be drawn with caution since these may have clinical consequences.

In another *in-vitro* study on Evo with attached VOL, Cripps et al. (2000) reported TED (ex-actuator), TDD (ex-spacer) and fine particle mass (stages 2-6) of 90 µg, ~53 µg (calculated by this author) and 43 µg (stages 2-6), respectively. This gives corresponding FPF as %TED (ex-actuator) and % of TDD (ex-spacer) of ~48% and ~82%. Their reported TED (ex-actuator), TDD (ex-spacer) and fine particle mass (stages 2-6) are ~5 µg (~5%), ~5 µg (~10%) and 2 µg (~5%) higher than those found

with the current study. Since percentages of these CQAs are within EMA limits (±15%), it can be concluded that two studies had effectively similar results.

These examples of *in-vitro* comparative studies show that %FPF alone may not correctly reflect on *in-vitro* performance of an inhaler treatment method which is being compared with others. The bronchodilator response is related to minimum effective amount of FPD of salbutamol that is available at the site of action rather than the fraction of TED containing FPD that reaches there. Clinical studies involving salbutamol dose-response such as methacholine challenge and dose titration identify this minimum required dose (Tomlinson et al., 2003; García-Arieta, 2014). Studies using FEV_1 as the endpoint reach plateau after achieving this minimum dose and further increase in the dose would not add additional relief. Hence minimum effective amount of salbutamol would bring about the desired relief. It is therefore prudent to use raw data to assess comparative performances of two or more MDIs without or with attached spacer while derived data can be used to assess individual performance efficiency of a treatment method.

6.2.7.6 MMAD and GSD

MMAD and GSD for Evo alone were relatively smaller than those obtained with attached spacer. Hence, the theory of spacer providing more time and space to generate finer particles (Fink, 2000) apparently seems to have not held ground here. However, the findings in this study for Evo are in concord with those of Cripps et al. (2000) who have also reported a lower MMAD with Evo alone (2.1 µm) than that found with VOL (2.4 µm).

Impactor mass (S0toF) profile (Figure 6.2.1) shows that with Evo, comparatively more TDD deposition was centred on group 2 stages (FPM) as compared to Evo+SP and had relatively smaller asymmetrical influences on the distribution profile from depositions on stages of groups 1 (fronting) (CPM) and 3 (tailing) (EPM). The size of MMAD found with spacer devices increased in order of VOL > ABLE > AERO. Also CPM, assessed as %TDD and as %S0toF, shows similar increasing order. Moreover, FPM was found in the same order but of decreasing amounts. This suggests that spacer shape and volume may have contributed to this sizing order. Larger volume and space provided by VOL resulted in lower proportions of CPM in TDD and S0toF which affected MMAD size accordingly. Nonetheless, MMAD and GSD obtained in present study lie between >2.5 to < 3 µm (range 0.23 µm) and > 1.5 to < 1.7 (range 0.12), respectively, and fall

within the desired size range for an optimal MDI performance (Section 2.3). Besides, the narrow window of GSD indicates that dispersion of aerodynamic particle size around their respective MMADs for these spacer combinations is similar.

6.2.7.7 Stage Groups

Efficacy and safety of an MDI is related to its APSD profile (Sections 2.3 & 3.3.4). The fractionation of TED/TDD from an MDI into various components of ACI could be useful in predicting its effectiveness and safety (Pritchard, 2001). Hence, APSD profiles have also been assessed as separate groups which may be representative of various regions of HRT (see Sections 3.3.4 & 5.2.4.10).

Statistical similarities of CPM, FPM and EPM (and FPD) observed between Evo alone and Evo+VOL suggest that the geometry and volume of this spacer correspond adequately with these MDI performance characteristics. CPM and EPM of Evo Vs Evo+AERO were statistically similar while their FPM (and FPD) was statistically different. Nevertheless, CPM and EPM of Evo Vs Evo+ABLE were statistically different even though their FPM (and FPD) was statistically similar. Also, CPM, FPM and EPM were not *in-vitro* equivalent between Evo Vs Evo+AERO and Vs Evo+ABLE. These findings indicate that stage grouped profile of Evo alone was not similar to either of them. In addition, EPM of Evo+VOL Vs Evo+AERO was statistically similar but significantly different Vs Evo+ABLE, the latter producing higher EPM. These findings however could not be reconciled with the theory of role of spacer volume since highest EPM was observed with Evo+ABLE rather than Evo+VOL. This would need further investigation given that VOL has larger diameter and axial length (Table 6.2.13), which theoretically allows more space to form finer particles.

6.2.7.8 Efficiency of dose delivery to ACI stages

The dose reaching ACI stages (S0toF) resembles that proportion of dose which enters respiratory system beyond oropharynx. Hence a ratio of S0toF to IP deposition could be used to predict the proportion of dose that would enter respiratory system post-pharynx. Evo alone delivered half of the dose to S0toF while this dose delivery ratio was increased to over 16 times with spacer; for Evo+VOL this dose delivery ratio was ~20 times more than that of Evo alone. This further supports use of spacers with Evo.

Relatively greater amount of dose was delivered to ACI stages (S0toF) for Evo+VOL than other spacers; the least dose delivered with AERO. However, the dose retained in

SP+IP was lower with Evo+VOL than that of IP deposition with Evo alone while it was higher with other two spacers, with consequent lower delivery to ACI stages.

6.2.7.9 Efficiency of respirable dose delivery (FPD/IP Ratio)

The ratio between lung (L) bioavailability of an inhaled drug to that of its total systemic (T) bioavailability would predict its efficacy and safety (Borgström, 1998). A higher L/T ratio would result in optimal efficacy with fewer side effects. Systemic bioavailability of inhaled drugs is a function of swallowing of inhaled dose impacted in oropharyngeal region and absorption from the upper HRT (laryngeal region). The ratio of FPD to IP deposition mimics L/T bioavailability and is considered an effective *in-vitro* means of determining efficiency of MDI dose delivery to the lungs (Wilkes et al., 2001). Evo when used alone was the least efficient in delivering FPD while this efficiency was the highest with Evo+VOL. FPD (Lung) to IP (throat) ratio decreased in order of Evo+VOL > Evo+ABLE > Evo+AERO > Evo (Table 6.2.7). The ratio of the respirable dose (FPD) to non-respirable dose (IP+CPM) followed similar trend of dose delivery efficiency. This trend was also reflected with spacer treatment methods (SP+IP+CPM). The ratios of respirable and non-respirable fractions suggest that spacers effectively retained large particle mass while significantly reducing IP deposition. However, their effect on reducing CPM deposition was not considerable. Hence the trend in decreasing ratio of FPD to IP+CPM Vs FPD to SP+IP+CPM, that is, the net respirable dose delivery decreased in order of Evo+VOL > Evo > Evo+ABLE > Evo+AERO. These findings suggest that Evo+VOL delivered more dose *in-vitro* in the respirable range than Evo alone and when attached to either ABLE or AERO. Further, FPD dose delivery of Evo alone was better than AERO and ABLE.

6.2.7.10 The dilemma of conflicting *in-vitro* results!

Differences and similarities amongst *in-vitro* studies conducted by different investigators are of a common occurrence. Even though same MDI products have been compared with each other in the present study, the differences in results have been found across regions and continents. This is because APSD measurements using ACI are a very sensitive technique with many potential sources of variability (Stein, 1999, 2008; Christopher et al., 2003; Mitchell and Nagel, 2003). Even results obtained from the same ACI equipment differ (Stein, 1999 & 2008). These potential sources of variability also include varying level of lab techniques, availability of supportive equipments and instruments, environmental conditions, methodology and protocols

(Christopher et al., 2003). With add-on devices such as spacers, this could introduce additional sources of variability between different laboratories. Hence, APSD data would reflect on these differences. Besides, differing objectives in assessing raw and derived data will have their own implications. Nevertheless, drastic differences would raise questions which would need to be investigated and answered.

Nonetheless, clinical studies have been reported where *in-vitro* performance of MDIs with and without attached spacers have not produced equivalent results yet these treatment methods were found to be bioequivalent (Dompeling et al., 2001; Barben et al., 2003; Dubus et al., 2003). Also, *in-vitro* studies are carried out in a controlled laboratory environment and the equipments used do not exactly replicate human respiratory system. Besides, *in-vitro* results are often higher than *in-vivo* results (Chan et al., 2014). Hence, *in-vitro* equivalence or its absence should not preclude *in-vivo* tests and clinical studies so as to allow researchers to see the wider picture. Conclusions based merely on *in-vitro* studies might be erroneous since at times these may not be predictive of clinical outcomes due to biological and behavioural variables.

6.2.8 Conclusions: *In-Vitro* Equivalence Studies of Ventolin Evohaler Without and With Spacer

The choice of an add-on device to be used with an MDI has been a popular research topic of interest lately. This is in part due to availability of a number of spacers and the intent of manufacturers to promote their products with a consequent emergence of studies to show their suitability to be used with a specific MDI. This independent study focussed on suitability of VOL, AERO and ABLE spacers as add-on device for Ventolin Evohaler.

TED (ex-actuator), FPD, MMAD and GSD of Ventolin Evohaler Vs EVO+VOL were *in-vitro* equivalent. Hence, VOL can be used as an add-on device for Evo when desired.

In-vitro equivalence with respect to TED (ex-actuator), FPD, MMAD and GSD were also observed between Evo and Evo+ABLE. Thus, ABLE spacer can be used as an add-on device with Ventolin Evohaler.

Evo alone and Evo+AERO were *in-vitro* equivalent with respect to TED (ex-actuator), MMAD and GSD only. FPD obtained with Evo+AERO was 5.5 µg lower than that of Evo alone and differed statistically and was not *in-vitro* equivalent. Based on this *in-vitro* information, AERO should not be recommended as an add-on device with

Ventolin Evohaler. Nevertheless, *in-vivo* studies with healthy volunteers have shown that AERO could be used with Ventolin Evohaler (Mazhar and Chrystyn, 2008).

Decisions on suitability of a given spacer should not be based on results of *in-vitro* studies only. In the event of failure to meet *in-vitro* equivalence, *in-vivo* studies should be conducted to support this or to ascertain its suitability.

The three spacers (VOL, AERO & ABLE) significantly reduced IP deposition to less than 6% and more than 79% of their TDD constituted FPD. Thus, these spacers were efficient in removing a large non-respirable fraction of TED.

Comparative performance of CQAs of MDI alone and with attached spacer should be assessed using raw data while derived data can be used for evaluating performance efficiency of a treatment method.

Findings of this study apply only to Ventolin Evohaler with VOL, AERO and ABLE and cannot be extended to other MDIs and spacers.

6.3 *In-Vivo* Equivalence of Ventolin Evohaler Without and With Spacers-Urinary Pharmacokinetic Studies

The objectives of this study are to determine bioavailability and *in-vivo* equivalence of Ventolin Evohaler alone and when used with spacers via urinary pharmacokinetic method (Hindle and Chrystyn, 1992). This will be achieved by comparing relative lung and total systemic bioavailability in healthy subjects. This will be complemented by charcoal blockade study to estimate lung deposition of inhaled salbutamol by preventing GI absorption of its swallowed fraction. These studies are of their first kind. Part of this work has been published.

6.3.1 Study Design

Study plan was implemented as per Section 3.4.5; subjects selected and trained as enumerated in Sections 3.4.3 and 3.4.4, respectively (Chapter 3). Subject cohort was the same as described in Section 5.3.1 (Chapter 5).

In summary, the clinical study consisted of two parts, each part with four sub-sets involving four treatment methods of salbutamol inhalation from Ventolin Evohaler (Evo), either the MDI alone or attached to one of the three spacers (SP): Volumatic (VOL), AeroChamber Plus (AERO) or Able (ABLE). In Part 1 Study, on separate study day one week apart, trained healthy subjects inhaled two separate puffs of salbutamol from one of these treatment methods selected randomly. Each dose discharged from Evo or into a spacer was inhaled using a slow vital capacity inhalation manoeuvre. The subjects exhaled to residual volume prior to actuating the dose, then took a slow deep inhalation over 5–10 seconds, followed by a 10 second breath hold. Subjects repeated this procedure for second actuation (Hindle et al., 1993). Before dosing Evo was primed and all spacers were washed in lukewarm mild detergent (equivalent to hand washing dishes), rinsed with water and left to air dry (Protocols 3.3.1 & 3.3.2; Sections 3.3.2.3 & 3.3.2.4; Chapter 3). Each single dose discharged into a spacer was inhaled within the first second of discharge into the spacer. All subjects voided their urine 0.5h pre-dosing. Thirty minutes after the start of each study dose inhalation subjects provided a urine sample (USAL0.5). They then pooled all their urine over the next 24h into a container (USAL24). In Part 2 Study, each subject repeated this study with the coadministration of activated charcoal by swallowing 100 mL of charcoal slurry immediately before and after completing two inhalations.

The pH and volume of each sample was recorded and samples were stored at –20°C before analysis. The pH values of the urine samples were all below pH 7, hence there was no variability due to passive tubular reabsorption (Hindle and Chrystyn, 1992).

After inhalation of the two study doses each spacer was rinsed with water to collect the residual dose.

6.3.2 Sample Analysis

Aqueous and urine samples were assayed for their salbutamol content using validated HPLC methods described in Chapter 4.

6.3.3 Statistical Analysis

Statistical analysis undertaken as per Section 3.4.7 (Chapter 3).

6.3.4 Results: *In-Vivo* Equivalence of Ventolin Evohaler Without and With Spacers

Volunteers' demographic characteristics are provided in Table 5.3.1 and Appendix 5.3.4.1 (Chapter 5). Summaries of USAL0.5 and USAL24 amounts post-dose (without and with charcoal blockade) are provided in Table 6.3.1 and Figure 6.3.1 to Figure 6.3.5. The table and figures also show salbutamol as active (USAL24Pre), active and sulphate conjugated (USAL24Post) and metabolised (USALMET) moieties excreted during 0.5h–24h period. Figure 6.3.6 shows comparative salbutamol urinary recovery profiles obtained post-inhalation without and with charcoal blockade. These recoveries of salbutamol as % nominal, % delivered and % recovered dose are given in Table 6.3.2 to Table 6.3.4. Urinary excretion data of individual subjects for Ventolin Evohaler is given in Appendices 5.3.4.2 and 5.3.4.5. Appendices 6.3.4.1 to 6.3.4.6 provide individual data for Evo+VOL, Evo+AERO and Evo+ABLE for both legs of the study. Table 6.3.5 to Table 6.3.8 provide data on *in-vivo* equivalence and comparative bioavailability of the four inhaled salbutamol treatment methods for both parts of the study. Table 6.3.9 compares charcoal blockade effect while *in-vitro* and *in-vivo* trends are shown in Table 6.3.10.

In Part 1 Study, relative lung bioavailability (USAL0.5NC) of Evo was not *in-vivo* equivalent to any of the three spacers attached to it (Table 6.3.1 & Table 6.3.5). USAL0.5NC of Evo attached to spacers (Evo+SP) was 2.5 times more than the amount of Evo alone and hence statistically significant. For total systemic bioavailability (USAL24NC), *in-vivo* equivalence as per EMA (2009) criteria was noted between Evo Vs Evo+VOL only although the three spacers had statistically similar total systemic bioavailability. However, these comparisons were *in-vivo* equivalent as per limits suggested by Parameswaran (1999). The excretion of active salbutamol (USAL24PreNC), its metabolites (USALMETnc) and total salbutamol (USAL24PostNC) were all *in-vivo* inequivalent between Evo Vs Evo+SP as per EMA (2009) criteria. Nevertheless, USAL24PreNC and USAL24PostNC (except Evo Vs Evo+AERO) were *in-vivo* equivalent on the basis of Parameswaran (1999) criteria. On the other hand, statistically similar bioavailability was only observed between Evo Vs Evo+SP and Evo Vs Evo+VOL for USAL24PreNC and USAL24PostNC, respectively.

The comparison of the two parts of the study is given in Table 6.3.9 (Figure 6.3.7). The mean paired differences between the amounts of salbutamol excreted in 0.5h were statistically similar between the two parts of the same treatment method (n=13).

Table 6.3.1. Mean salbutamol excreted in urine from Ventolin Evohaler without and with spacer.

Treatment (n = 13)	**USAL0.5**		**USAL24†**		**USAL24Pre**		**USAL24Post**		**USALMET**	
	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD
Part 1 Study (without charcoal blockade)										
Ventolin*	5.7	1.9	100.7	15.7	58.4	18.3	95.0	16.6	36.6	15.6
Evo + VOL	16.4	8.2	97.3	12.7	62.8	11.8	85.3	12.5	22.5	8.9
Evo + AERO	14.8	7.4	84.6	25.8	57.1	19.6	71.9	21.5	14.8	4.5
Evo + ABLE	14.4	5.4	89.7	21.0	61.9	15.3	75.3	16.4	13.4	4.8
Part 2 Study (with charcoal blockade)										
Ventolin*	5.3	2.5	29.8	5.3	14.3	5.3	24.5	4.8	10.2	5.1
Evo + VOL	14.8	4.9	68.9	15.1	39.0	10.0	54.1	11.8	15.1	6.3
Evo + AERO	13.8	6.7	64.6	17.6	39.6	10.7	50.8	11.8	11.2	4.4
Evo + ABLE	14.1	5.8	62.0	19.6	35.0	11.1	47.9	14.1	12.9	5.7

* Ventolin Evohaler; † TRD = USAL24; SD = Standard Deviation

Between spacers, USAL0.5NC and USAL24NC were not *in-vivo* equivalent as per EMA (2009) despite having statistically similar bioavailability (Table 6.3.1 & Table 6.3.6). These parameters were, however, within the limits suggested by Parameswaran

(1999). Similar results were observed for USAL24PreNC and USAL24PostNC. Nevertheless, USALMETnc was statistically different between Evo+VOL Vs Evo+AERO and Vs Evo+ABLE which also failed to meet *in-vivo* equivalence criteria of Parameswaran (1999).

Table 6.3.2. Mean salbutamol excretion in urine from Ventolin Evohaler without and with the spacer, expressed as % of Nominal Dose.

Treatment Method (n = 13)	**USAL0.5**		**USAL24†**		**USAL24Pre**		**USAL24Post**		**USALMET**	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Ventolin*	2.9	0.9	50.3	7.9	29.2	9.1	47.5	8.3	18.3	7.8
Evo + VOL	8.2	4.1	48.7	6.3	31.4	5.9	42.6	6.3	11.2	4.5
Evo + AERO	7.4	3.7	42.3	12.9	28.6	9.8	36.0	10.7	7.4	2.3
Evo + ABLE	7.2	2.7	44.9	10.5	31.0	7.6	37.6	8.2	6.7	2.4
Part 2 Study (with charcoal blockade)										
Ventolin*	2.6	1.2	14.9	2.7	7.2	2.7	12.3	2.4	5.1	2.5
Evo + VOL	7.4	2.5	34.5	7.5	19.5	5.0	27.1	5.9	7.5	3.2
Evo + AERO	6.9	3.4	32.3	8.8	19.8	5.4	25.4	5.9	5.6	2.2
Evo + ABLE	7.0	2.9	31.0	9.8	17.5	5.5	24.0	7.1	6.4	2.9

* Ventolin Evohaler; † TRD = USAL24; SD = Standard Deviation

In Part 2 Study with charcoal blockade, none of these PK parameters were either *in-vivo* equivalent or had statistically similar bioavailability between Evo Vs Evo+SP. Exceptions were for USALMETc; Evo Vs Evo+AERO were *in-vivo* equivalent as per Parameswaran (1999) criteria which also showed similar bioavailability along with Evo Vs Evo+ABLE (Table 6.3.1 & Table 6.3.7). The USAL0.5C amount of Evo+SP was over 2.5 times more than Evo alone and was statistically significant. On the other hand, charcoal prevented GI absorption of swallowed salbutamol from Evo thereby significantly reducing its USAL24C by more than half of Evo+SP. Similar *in-vivo* inequivalent trend in other pharmacokinetic (PK) parameters was observed between Evo+SP as per EMA (2009) criteria. On the contrary, these PK parameters were *in-vivo* equivalent and had statistically similar bioavailability; exception of both measurements being between Evo+VOL Vs Evo+AERO for USALMETc (Table 6.3.1 & Table 6.3.8).

Table 6.3.3. Mean salbutamol excretion in urine from Ventolin Evohaler without and with spacers, expressed as % of estimated Delivered Dose.

Treatment Method (n = 13)	USAL0.5		USAL24†		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Ventolin*	3.6	1.2	62.7	9.4	36.3	11.0	59.1	10.0	22.8	9.7
Evo + VOL	16.0	8.4	93.2	2.1	60.2	8.4	81.6	5.3	21.4	7.2
Evo + AERO	17.9	11.3	93.5	6.7	62.5	10.3	79.8	7.3	17.3	5.4
Evo + ABLE	14.6	3.7	93.0	4.0	64.3	5.6	78.3	3.0	14.1	5.5
Part 2 Study (with charcoal blockade)										
Ventolin*	3.3	1.5	18.7	3.4	9.0	3.4	15.4	3.1	6.4	3.2
Evo + VOL	14.3	4.7	66.1	12.8	37.4	8.8	51.8	9.7	14.4	5.8
Evo + AERO	15.3	6.5	72.6	14.8	44.6	9.8	57.3	9.8	12.7	4.7
Evo + ABLE	14.6	4.9	64.9	14.9	36.8	9.2	50.3	10.4	13.5	5.1

* Ventolin Evohaler; † TRD = USAL24; SD = Standard Deviation

Table 6.3.4. Mean salbutamol excretion in urine from Ventolin Evohaler without and with spacers, expressed as % of Recovered Dose.

Treatment Method (n = 13)	USAL0.5		USAL24†		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Ventolin*	5.9	2.4	-	-	57.7	14.6	94.1	2.4	36.3	14.4
Evo + VOL	17.3	9.2	-	-	64.6	8.7	87.5	5.7	23.0	7.9
Evo + AERO	19.4	12.4	-	-	66.7	8.8	85.5	6.4	18.8	7.0
Evo + ABLE	15.7	3.6	-	-	69.2	5.9	84.3	3.6	15.2	6.1
Part 2 Study (with charcoal blockade)										
Ventolin*	17.7	7.1	-	-	48.7	16.0	82.3	7.1	33.7	14.6
Evo + VOL	21.3	5.1	-	-	56.6	7.4	78.7	5.1	22.1	8.2
Evo + AERO	20.4	5.5	-	-	61.5	7.4	79.6	5.5	18.1	7.9
Evo + ABLE	22.0	3.7	-	-	56.8	8.9	78.0	3.7	21.2	7.6

* Ventolin Evohaler; † TRD = USAL24; SD = Standard Deviation

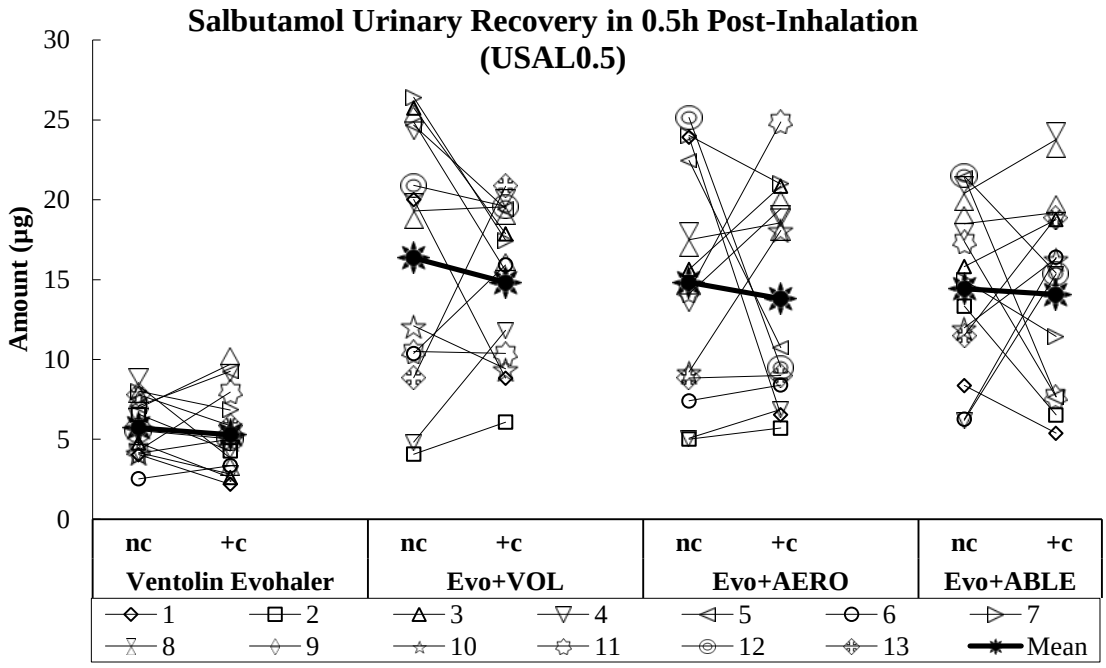


Figure 6.3.1. Comparative salbutamol urinary excretion at 0.5h post-inhalation without and with charcoal ingestion.

Numerals represent individual volunteers. nc = no charcoal ingestion; +c = with charcoal ingestion.

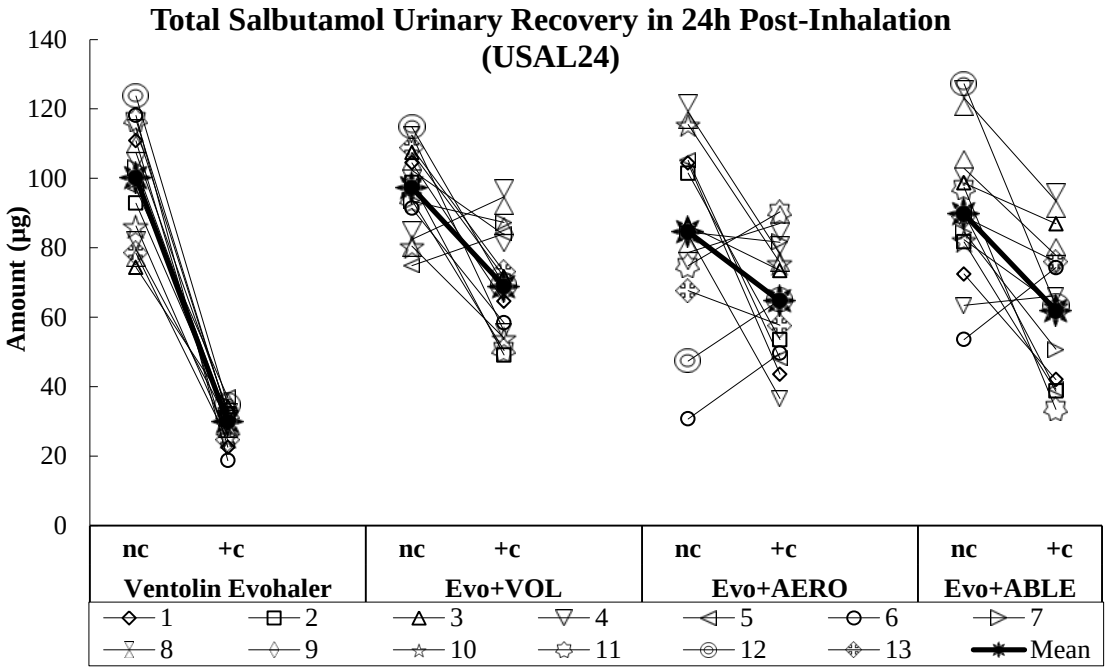


Figure 6.3.2. Comparative total salbutamol urinary excretion during 24h post-inhalation without and with charcoal ingestion.

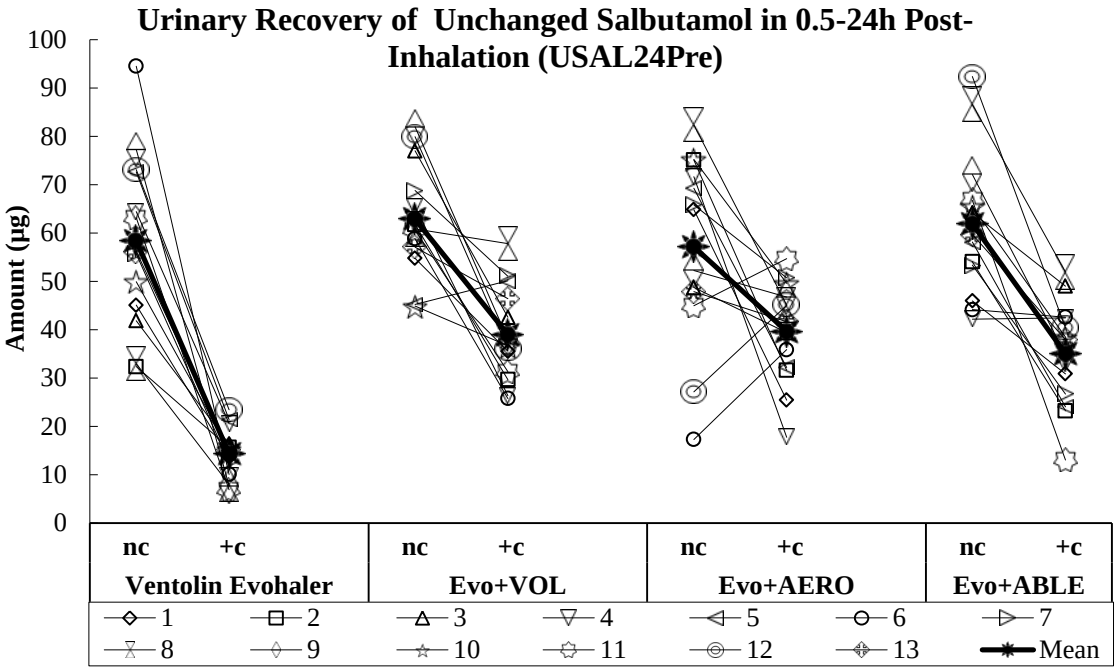


Figure 6.3.3. Comparative unchanged salbutamol urinary excretion during 0.5-24h post-inhalation without and with charcoal ingestion.

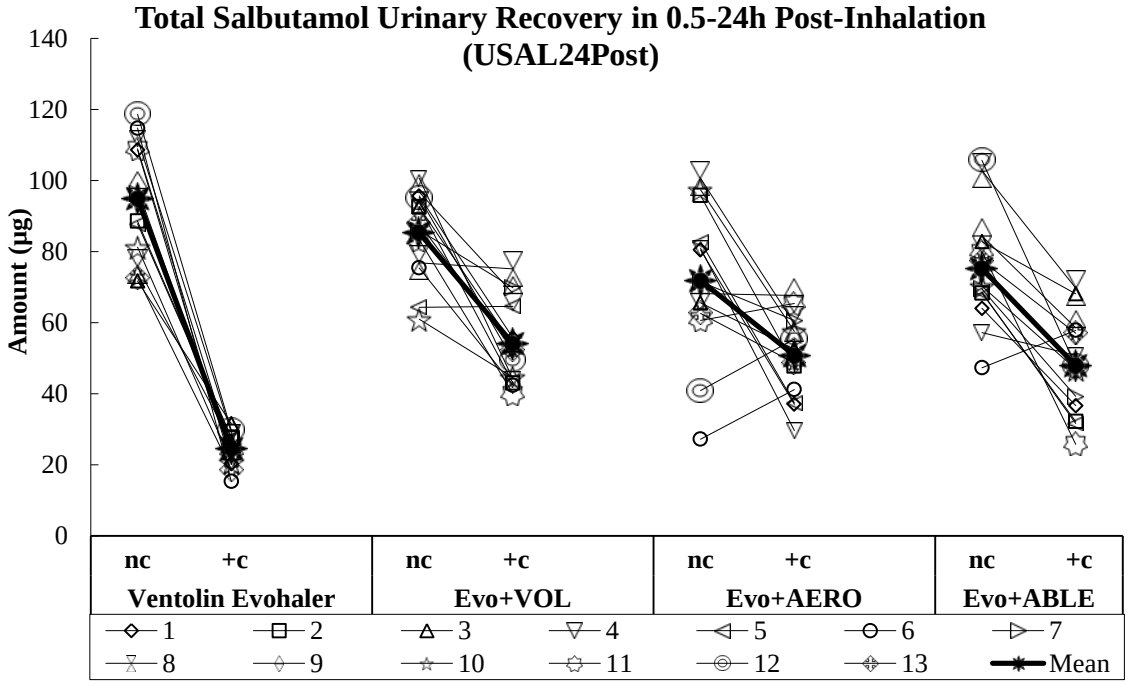


Figure 6.3.4. Comparative total salbutamol urinary excretion during 0.5-24h post-inhalation without and with charcoal ingestion.

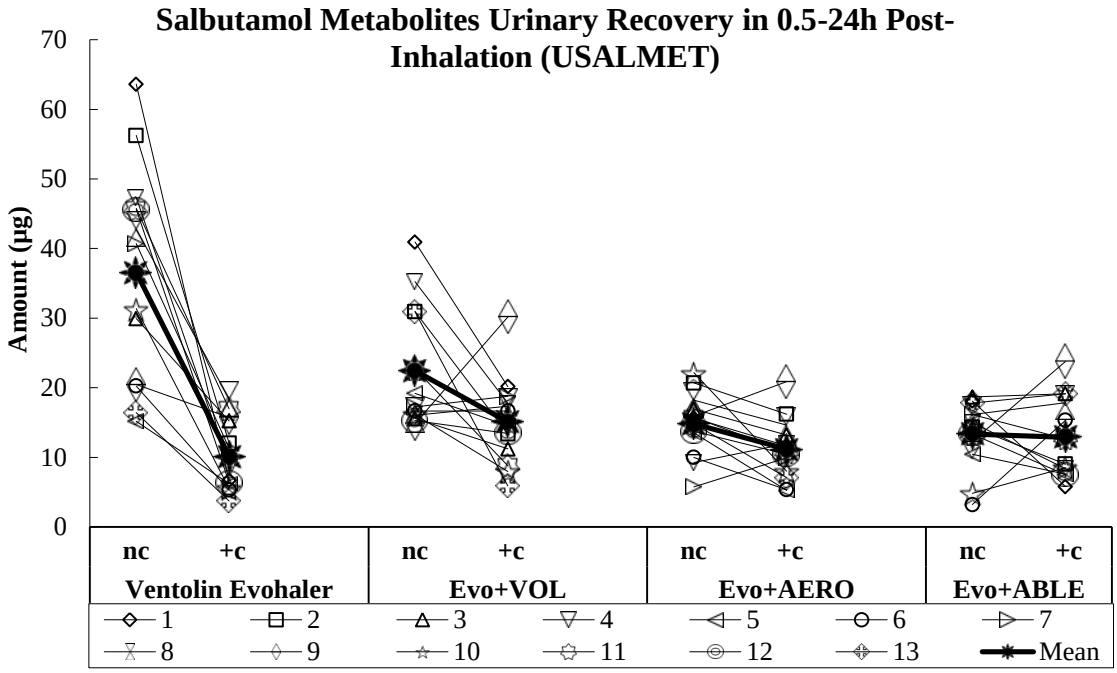


Figure 6.3.5. Comparative salbutamol metabolites urinary excretion during 0.5-24h post-inhalation without and with charcoal ingestion.

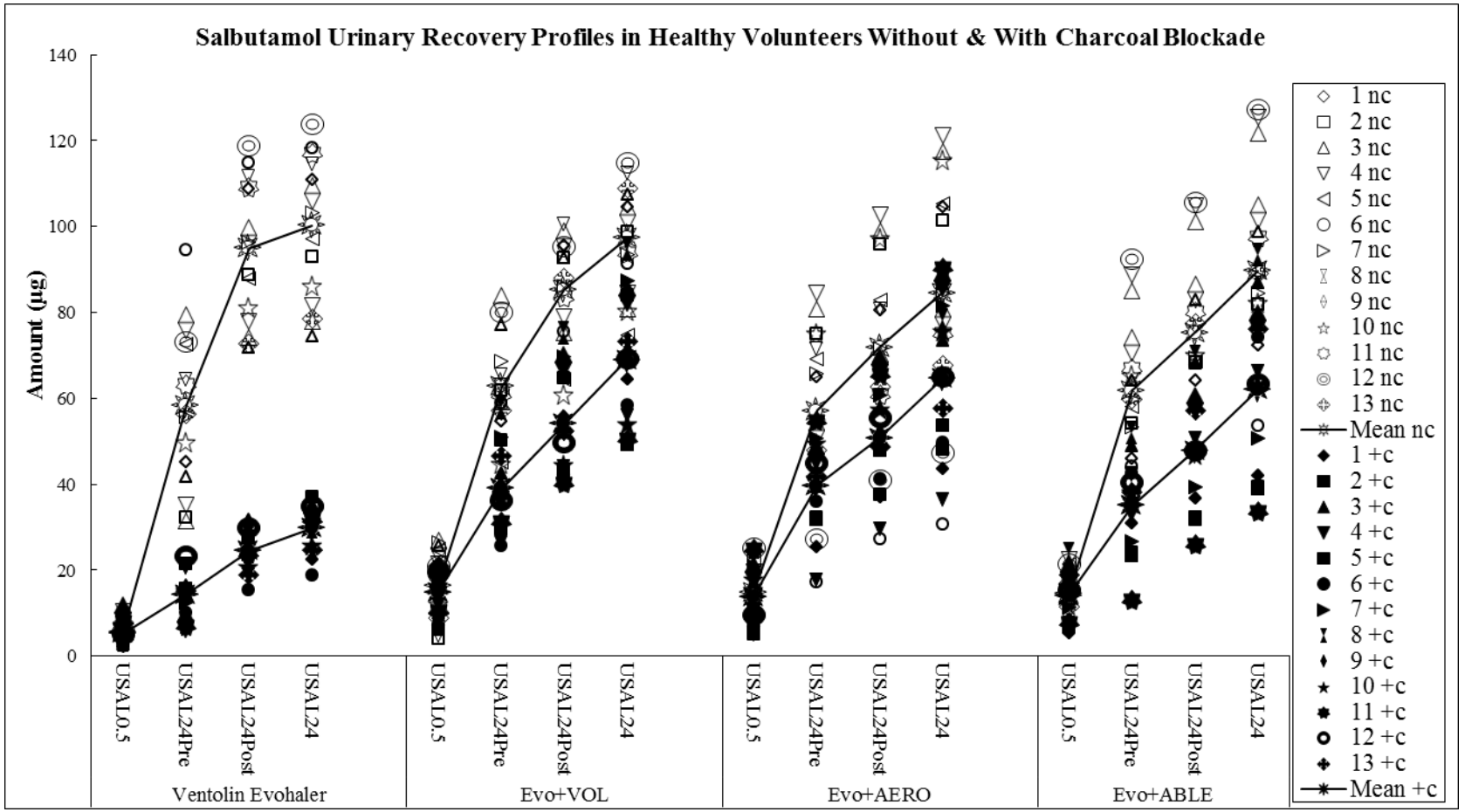


Figure 6.3.6. Comparative salbutamol urinary recovery profiles obtained post-inhalation without and with charcoal ingestion.

Numerals represent individual volunteers. nc = no charcoal ingestion; +c = with charcoal ingestion.

Table 6.3.5. *In-Vivo* Equivalence and Statistical Significance of Ventolin Evohaler Vs Evo+SP salbutamol urinary excretion without charcoal blockade (Part 1 Study).

Parameter	Multiple Comparisons	Mean Ratio	90% CI		*p* value	*In-Vivo* Equivalence		Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
	Ventolin Evohaler MDI Vs		LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
USAL0.5 NC	Evo + Volumatic	0.39	0.31	0.49	<0.0001	No	No	-10.64	-14.17	-7.12	<0.0001	No
	Evo + AeroChamber Plus	0.42	0.33	0.53	<0.0001	No	No	-9.11	-12.63	-5.58	<0.0001	No
	Evo + ABLE	0.41	0.32	0.51	<0.0001	No	No	-8.72	-12.24	-5.19	<0.0001	No
USAL24 NC	Evo + Volumatic	1.02	0.86	1.22	0.814	Yes	Yes	2.92	-13.78	19.61	0.725	Yes
	Evo + AeroChamber Plus	1.24	1.04	1.47	0.044	No	Yes	15.67	-1.03	32.36	0.065	Yes
	Evo + ABLE	1.13	0.95	1.35	0.234	No	Yes	10.56	-6.13	27.25	0.208	Yes
USAL24Pre NC	Evo + Volumatic	0.90	0.72	1.13	0.448	No	Yes	-4.45	-24.31	15.41	1.000	Yes
	Evo + AeroChamber Plus	1.05	0.84	1.33	0.706	No	Yes	1.68	-18.18	21.54	1.000	Yes
	Evo + ABLE	0.92	0.73	1.16	0.565	No	Yes	-3.53	-23.39	16.33	1.000	Yes
USAL24Post NC	Evo + Volumatic	1.11	0.94	1.31	0.301	No	Yes	9.69	-4.61	23.98	0.178	Yes
	Evo + AeroChamber Plus	1.37	1.16	1.62	0.003	No	No	23.08	8.78	37.37	0.002	No
	Evo + ABLE	1.27	1.08	1.50	0.021	No	Yes	19.70	5.41	34.00	0.008	No
USALMET nc	Evo + Volumatic	1.58	1.22	2.05	0.005	No	No	14.14	7.47	20.82	<0.0001	No
	Evo + AeroChamber Plus	2.37	1.83	3.07	<0.0001	No	No	21.79	15.12	28.47	<0.0001	No
	Evo + ABLE	2.74	2.12	3.56	<0.0001	No	No	23.23	16.56	29.91	<0.0001	No

[a] EMA, 2009; [b] Parameswaran, 1999; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit; NC or nc = No Charcoal

Table 6.3.6. *In-Vivo* Equivalence and Statistical Significance of salbutamol urinary excretion post-inhalation from Ventolin Evohaler with spacer without charcoal blockade (Part 1 Study).

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p value*	*In-Vivo* Equivalence		Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
				LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
USAL0.5 NC	Evo+VOL	Evo+AERO	1.08	0.86	1.36	0.562	No	Yes	1.54	-1.99	5.06	0.382	Yes
		Evo+ABLE	1.05	0.83	1.32	0.737	No	Yes	1.93	-1.60	5.45	0.275	Yes
	Evo+AERO	Evo+ABLE	0.97	0.77	1.22	0.806	No	Yes	0.39	-3.13	3.91	0.824	Yes
USAL24 NC	Evo+VOL	Evo+AERO	1.21	1.02	1.44	0.073	No	Yes	12.75	-3.94	29.45	0.130	Yes
		Evo+ABLE	1.10	0.93	1.31	0.336	No	Yes	7.64	-9.05	24.34	0.359	Yes
	Evo+AERO	Evo+ABLE	0.91	0.77	1.09	0.390	No	Yes	-5.11	-21.80	11.59	0.539	Yes
USAL24Pre NC	Evo+VOL	Evo+AERO	1.17	0.93	1.47	0.258	No	Yes	5.74	-8.55	20.02	0.421	Yes
		Evo+ABLE	1.03	0.82	1.29	0.853	No	Yes	0.92	-13.36	15.21	0.896	Yes
	Evo+AERO	Evo+ABLE	0.88	0.70	1.10	0.342	No	Yes	-4.81	-19.10	9.47	0.499	Yes
USAL24Post NC	Evo+VOL	Evo+AERO	1.24	1.05	1.46	0.038	No	Yes	13.39	-0.91	27.69	0.066	Yes
		Evo+ABLE	1.15	0.97	1.35	0.178	No	Yes	10.02	-4.28	24.31	0.164	Yes
	Evo+AERO	Evo+ABLE	0.93	0.78	1.09	0.441	No	Yes	-3.37	-17.67	10.93	0.635	Yes
USALMET nc	Evo+VOL	Evo+AERO	1.50	1.16	1.95	0.012	No	No	7.65	0.98	14.33	0.026	No
		Evo+ABLE	1.74	1.34	2.26	0.001	No	No	9.09	2.42	15.77	0.009	No
	Evo+AERO	Evo+ABLE	1.16	0.89	1.50	0.346	No	Yes	1.44	-5.23	8.11	0.664	Yes

[a] EMA, 2009; [b] Parameswaran, 1999; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit; NC or nc = No Charcoal

Table 6.3.7. *In-Vivo* Equivalence and Statistical Significance of Evohaler Vs Evo+SP salbutamol urinary excretion post-inhalation with charcoal blockade (Part 2 Study).

Parameter	Multiple Comparisons	Mean Ratio	90% CI		*p* value	*In-Vivo* Equivalence		Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
	Ventolin Evohaler MDI Vs		LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
USAL0.5C	Evo + Volumatic	0.34	0.26	0.44	<0.0001	No	No	-9.54	-13.16	-5.92	<0.0001	No
	Evo + AeroChamber Plus	0.39	0.30	0.50	<0.0001	No	No	-8.53	-12.15	-4.91	<0.0001	No
	Evo + ABLE	0.37	0.29	0.48	<0.0001	No	No	-8.77	-12.39	-5.15	<0.0001	No
USAL24C	Evo + Volumatic	0.43	0.37	0.51	<0.0001	No	No	-39.14	-50.05	-28.23	<0.0001	No
	Evo + AeroChamber Plus	0.47	0.40	0.55	<0.0001	No	No	-34.86	-45.77	-23.94	<0.0001	No
	Evo + ABLE	0.50	0.42	0.58	<0.0001	No	No	-32.18	-43.09	-21.27	<0.0001	No
USAL24PreC	Evo + Volumatic	0.35	0.28	0.44	<0.0001	No	No	-24.68	-32.18	-17.18	<0.0001	No
	Evo + AeroChamber Plus	0.35	0.28	0.44	<0.0001	No	No	-25.32	-32.82	-17.82	<0.0001	No
	Evo + ABLE	0.40	0.32	0.51	<0.0001	No	No	-20.68	-28.18	-13.18	<0.0001	No
USAL24PostC	Evo + Volumatic	0.45	0.39	0.53	<0.0001	No	No	-29.60	-37.75	-21.45	<0.0001	No
	Evo + AeroChamber Plus	0.49	0.42	0.57	<0.0001	No	No	-26.33	-34.48	-18.18	<0.0001	No
	Evo + ABLE	0.52	0.45	0.61	<0.0001	No	No	-23.41	-31.56	-15.25	<0.0001	No
USALMETc	Evo + Volumatic	0.65	0.51	0.83	0.004	No	No	-4.92	-8.21	-1.63	0.004	No
	Evo + AeroChamber Plus	0.87	0.68	1.10	0.326	No	Yes	-1.01	-4.30	2.28	0.536	Yes
	Evo + ABLE	0.77	0.60	0.97	0.069	No	No	-2.72	-6.01	0.57	0.102	Yes

[a] EMA, 2009; [b] Parameswaran, 1999; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit; C or c = Charcoal

Table 6.3.8. *In-Vivo* Equivalence and Statistical Significance of salbutamol urinary excretion post-inhalation from Ventolin Evohaler with spacer with charcoal ingestion (Part 2 Study).

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p value*	*In-Vivo* Equivalence		Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
				LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
USAL0.5C	Evo+VOL	Evo+AERO	1.14	0.88	1.47	0.406	No	Yes	1.01	-2.61	4.63	0.574	Yes
		Evo+ABLE	1.09	0.84	1.41	0.574	No	Yes	0.77	-2.85	4.39	0.670	Yes
	Evo+AERO	Evo+ABLE	0.96	0.74	1.24	0.786	No	Yes	-0.25	-3.87	3.37	0.891	Yes
USAL24C	Evo+VOL	Evo+AERO	1.08	0.92	1.27	0.408	No	Yes	4.28	-6.63	15.20	0.431	Yes
		Evo+ABLE	1.14	0.98	1.34	0.161	No	Yes	6.96	-3.95	17.87	0.204	Yes
	Evo+AERO	Evo+ABLE	1.06	0.90	1.24	0.557	Yes	Yes	2.68	-8.23	13.59	0.622	Yes
USAL24PreC	Evo+VOL	Evo+AERO	0.99	0.79	1.25	0.970	No	Yes	-0.64	-8.14	6.86	0.864	Yes
		Evo+ABLE	1.15	0.91	1.44	0.321	No	Yes	4.00	-3.51	11.50	0.287	Yes
	Evo+AERO	Evo+ABLE	1.15	0.92	1.45	0.303	No	Yes	4.63	-2.87	12.14	0.218	Yes
USAL24PostC	Evo+VOL	Evo+AERO	1.07	0.92	1.25	0.460	Yes	Yes	3.27	-4.88	11.42	0.422	Yes
		Evo+ABLE	1.15	0.99	1.35	0.126	No	Yes	6.19	-1.96	14.35	0.132	Yes
	Evo+AERO	Evo+ABLE	1.08	0.92	1.26	0.418	No	Yes	2.93	-5.23	11.08	0.471	Yes
USALMETc	Evo+VOL	Evo+AERO	1.34	1.05	1.70	0.048	No	No	3.91	0.62	7.20	0.021	No
		Evo+ABLE	1.18	0.93	1.50	0.252	No	Yes	2.20	-1.09	5.49	0.184	Yes
	Evo+AERO	Evo+ABLE	0.88	0.69	1.12	0.386	No	Yes	-1.71	-5.00	1.58	0.299	Yes

[a] EMA, 2009; [b] Parameswaran, 1999; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit; C or c = Charcoal

Table 6.3.9. Statistical comparison of salbutamol urinary excretion between Parts 1 and 2 studies.

Parameter [nc Vs (+c)]	**Treatment Method**	**Mean paired Difference**	**95% CI**		***t value***	***p value***	**Statistical Similarity**
			LL	**UL**			
USAL0.5	Ventolin*	0.43	-0.99	1.85	.661	0.521	Yes
	Evo+VOL	1.53	-2.70	5.76	.789	0.445	Yes
	Evo+AERO	1.01	-4.32	6.34	.412	0.687	Yes
	Evo+ABLE	0.37	-4.13	4.88	.180	0.860	Yes
USAL24	Ventolin*	76.19	66.01	86.37	16.309	<0.0001	No
	Evo+VOL	28.42	15.26	41.58	4.705	0.001	No
	Evo+AERO	19.95	1.79	38.11	2.394	0.034	No
	Evo+ABLE	27.74	12.88	42.59	4.068	0.002	No
USAL24Pre	Ventolin*	44.05	32.70	55.40	8.458	<0.0001	No
	Evo+VOL	23.83	14.16	33.49	5.373	<0.0001	No
	Evo+AERO	17.45	2.89	32.01	2.612	0.023	No
	Evo+ABLE	26.90	16.93	36.87	5.877	<0.0001	No
USAL24Post	Ventolin*	70.47	59.71	81.24	59.71	<0.0001	No
	Evo+VOL	31.19	20.43	41.95	20.43	<0.0001	No
	Evo+AERO	21.07	6.19	35.95	6.19	0.009	No
	Evo+ABLE	27.37	16.39	38.35	16.39	<0.0001	No
USALMET	Ventolin*	26.42	17.17	35.68	6.222	<0.0001	No
	Evo+VOL	7.36	0.58	14.14	2.366	0.036	No
	Evo+AERO	3.62	0.46	6.78	2.493	0.028	No
	Evo+ABLE	0.47	-3.71	4.65	.244	0.812	Yes

* Ventolin Evohaler

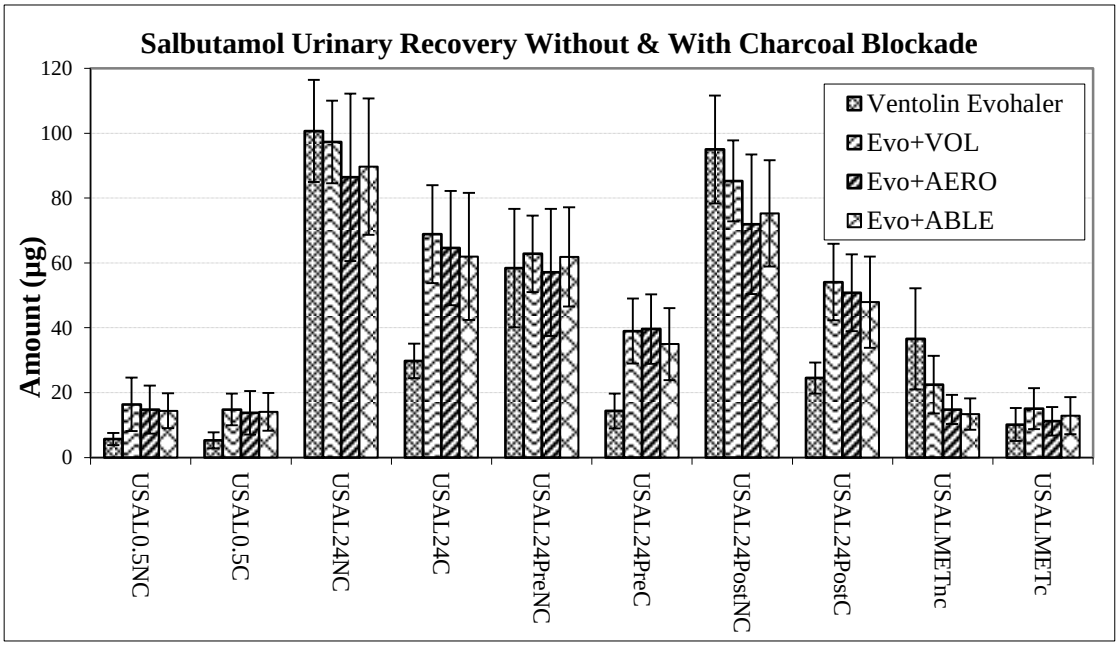


Figure 6.3.7. Comparison of salbutamol urinary excretion from Ventolin Evohaler treatment methods between Parts 1 and 2 studies.

Table 6.3.10. *In-vitro* and *in-vivo* correlation trends between Evo and Evo+SP performance metrics and salbutamol urinary excretion post-inhalation.

Parameter	Ventolin*	Evo+VOL	Evo+AERO	Evo+ABLE	Trend (in decreasing order)
	µg	µg	µg	µg	
FPD	78.4	78.1	67.5	74.0	Evo > Evo+VOL > Evo+ABLE > Evo+AERO
%FPF (%TDD)	44.4	82.2	79.2	80.3	Evo+VOL > Evo+ABLE > Evo+AERO > Evo
S0toF	87.9	89.4	80.3	87.1	Evo+VOL > Evo > Evo+ABLE > Evo+AERO
USAL0.5NC	5.7	16.4	14.8	14.4	Evo+VOL > Evo+AERO > Evo+ABLE > Evo
USAL0.5C	5.3	14.8	13.8	14.1	Evo+VOL > Evo+ABLE > Evo+AERO > Evo
IP+CPM	98.2	16.8	17.9	18.2	Evo > Evo+ABLE > Evo+AERO > Evo+VOL
IP	88.8	5.5	5.0	5.1	Evo > Evo+VOL > Evo+ABLE > Evo+AERO
USAL24PreNC	58.4	62.8	57.1	61.9	Evo+VOL > Evo+ABLE > Evo > Evo+AERO
USAL24PreC	14.3	39.0	39.6	35.0	Evo+AERO > Evo+VOL > Evo+ABLE > Evo
USAL24PostNC	95.0	85.3	71.9	75.3	Evo > Evo+VOL > Evo+ABLE > Evo+AERO
USAL24PostC	24.5	54.1	50.8	47.9	Evo+VOL > Evo+AERO > Evo+ABLE > Evo
TDD (ACI)	176.6	94.9	85.3	92.3	Evo > Evo+VOL > Evo+ABLE > Evo+AERO
TDD (NC)	160.6	104.3	89.3	95.9	Evo > Evo+VOL > Evo+ABLE > Evo+AERO
TDD (C)	159.2	104.0	87.8	93.7	Evo > Evo+VOL > Evo+ABLE > Evo+AERO
USAL24NC	100.7	97.3	84.6	89.7	Evo > Evo+VOL > Evo+ABLE > Evo+AERO
USAL24C	29.8	68.9	64.6	62.0	Evo+VOL > Evo+AERO > Evo+ABLE > Evo

* Ventolin Evohaler

6.3.5 Discussion

Bronchodilator MDIs are widely prescribed for respiratory ailments even though many patients find it difficult to use them correctly (Levy et al., 2013 & 2016; Sanchis et al. 2016), and in particular during acute asthma attack. The problem of MDI technique may be of greater concern for paediatric and elderly patients. A common issue is poor coordination between actuation of the MDI and inhalation of the medication (Crompton, 1982; Rau, 2006; Al-Showair et al., 2007; Melani et al., 2011; Roche et al., 2013), which persists even after training (Crompton et al., 2006). The use of spacers with MDIs has been found to ease this coordination difficulty by allowing few seconds after inhaler is actuated and the patient starts inhaling (Mitchell and Nagel, 2007; Nikander et al., 2014). Compatible spacers, therefore, may potentially improve drug delivery from MDIs (Newman, 1991; Chrystyn and Price, 2009; Levy et al., 2013).

The results of this work indicate that the three spacers used with Evo significantly increased lung deposition (USAL0.5NC) as compared to Evo alone; this increase being over 2.5-fold (Table 6.3.1). Hence, relative lung bioavailability of Evo was higher with the three spacers than Evo alone. This increase in dose delivery with Evo+SP was consistent in both legs of the study, i.e., without and with charcoal blockade, reaffirming that the urinary excretion of inhaled salbutamol in the first 0.5h originated from the proportion of the dose deposited in the lungs.

The statistical similarity of relative lung bioavailability (USAL0.5NC) between Evo+SP treatment methods in Part 1 Study suggests that lung deposition was not affected by either the spacer dimension or volume (Table 6.3.1 & Table 6.3.6). This is further supported by the results of Part 2 Study for USAL0.5C (Table 6.3.1 & Table 6.3.8).

In Part 1 Study, total systemic bioavailability (USAL24NC) was statistically similar, indicating that the total systemic delivery from either Evo alone or with spacer was not affected by the treatment method. This suggests that both methods of treatment may have similar pattern of systemic effects.

In Part 2 Study with charcoal blockade, the significant differences between the treatment methods for total systemic bioavailability (USAL24C) indicate a correlation existed between USAL0.5C and USAL24C within them; USAL24C amount of Evo alone was 5.6 times of USAL0.5C while within Evo+SP, this was 4.4 to 4.7 times.

USAL0.5C constituted 18% for Evo alone and 20-22% for Evo+SP of the total systemic bioavailability (Table 6.3.4). Interestingly, USAL0.5NC represented 16-19% of USAL24NC in Part 1 Study. These findings for Evo+SP in both legs of study suggest that major contribution to total systemic bioavailability was derived from the lung deposition. The 3-fold difference in USAL0.5 between the two parts of the study highlights the smaller contribution of USAL0.5NC to USAL24NC due to GI absorption of swallowed proportion of salbutamol.

In Part 1 Study, USAL24NC of spacer treatment methods was 1.3 to 1.5 folds more than that of USAL24C of Part 2 Study and these were significantly different between the two parts. USAL24NC represented 42 to 49 % and USAL24C 31 to 35 % of the nominal dose (Table 6.3.2 and Table 6.3.4). When normalised for TDD, these values were 93 to 94 % and 65 to 73 %, respectively (Table 6.3.9). These results suggest that total systemic bioavailability (USAL24) with spacer treatment method had more than 65% contribution from the lung deposition. This further highlights the dose delivery efficiency of the three spacer treatment methods.

Total systemic bioavailability (USAL24) of spacer treatment methods was statistically similar in both legs of the study (Table 6.3.6 and Table 6.3.8). This indicates consistency in dose delivery from spacers. This also highlights that the subjects may have greater control over inhaling the dispensed dose from Evo when used with any one of the three spacers.

In Part 1 Study, recovery of statistically similar unchanged salbutamol (USAL24PreNC) suggests that equivalent pharmacologically active salbutamol was bioavailable for continued bronchodilation from Evo alone and with the three spacers (Table 6.3.5). However, in Part 2 Study, this was not the case and goes against this hypothesis (Table 6.3.7). With Evo+SP treatment methods, USAL24PreNC was 1.4 to 1.8 folds more than that of USAL24PreC. Further, the former constituted 57 to 69% and the latter 57 to 62% of the active salbutamol (Table 6.3.4). On the other hand, USAL24PreC was 1/4th of USAL24PreNC for Evo alone and indicates that only 25% of the USAL24PreNC was bioavailable for continued relief from the dose deposited in the lungs. Interestingly, these amounts of USAL24PreNC and USAL24PreC represented respectively 58% and 49% of total systemic bioavailability. These findings suggest that with Evo alone, a fraction of active salbutamol (USAL24PreNC) may have bypassed enterohepatic metabolism and that this may have also entered into systemic circulation from the lungs

over 24h post-inhalation (Ward et al., 2000; Chrystyn, 2001). This is evident from 4-fold higher recovery of USAL24PreNC than USAL24PreC and reflected on recoveries of respective metabolites (USALMET) in the two legs of the study. Hence, the apparent statistical similarity of the USAL24PreNC of Evo MDI Vs Evo+SP may be misleading in terms of their continued equal relief and effectiveness. The inspection of results rather suggest that Evo+SP may be more effective in providing relief with inhaled salbutamol spread over the dosing interval. However, this should be understood with caution since the efficacy indicators such as FEV_1 do not show further improvement after achieving endpoint and increasing salbutamol dose may not change the plateau, i.e., after achieving minimum effective dose, further improvement in spirometry may not be observed. Nevertheless, USAL24Pre has been measured over 0.5 to 24 hour post-inhalation, hence it can be safely assumed that there may have been a prolonged presence of active salbutamol in the lungs for continued relief.

Chege and Chrystyn (1994) reported the use of VOL with Ventolin CFC and generic salbutamol MDI (Baker Norton, UK) employing 4 puffs of 100 µg each. They found that these two treatment methods delivered similar amounts of drug to the lungs and the total systemic absorption was the same. The results of current work for Evo+SP in both legs of study are consistent with their findings. Their reported amounts of USAL0.5 and USAL24 for Ventolin CFC attached to VOL were 5.3% and 28.7% of the nominal dose. The respective amounts reported in this thesis for Evo+VOL are however higher by factors of 1.5 and 1.7 to theirs.

Silkstone et al. (2002a) reported the amounts for USAL0.5 of 12.6 µg and 27.1 µg for Ventolin CFC alone and attached to VOL for 5 puffs which are equivalent of 2.5% and 5.4% of the nominal dose. Their respective USAL24 amounts were 287.0 µg and 198.1 µg which are equivalent of 57.4% and 39.6% of the nominal dose. The USAL0.5 amounts when normalised for the dose available for inhalation were equivalent to 2.9% and 9.2%, respectively, for the two treatment methods. The normalised values of USAL24 for these treatments were 64.9% and 67.7%, respectively. The corresponding USAL0.5 normalised values for Evo alone and Evo+VOL reported in this thesis are 3.6% and 16% and show respective ratios of 1.24 and 1.74 to their results. These results suggest that relative lung delivery of Ventolin Evohaler was better than that of Ventolin CFC with both treatment methods. Also, normalised values for USAL24 reported in the present work are 62.7% and 93% for the two treatment methods which have ratios of 0.97 and 1.38 to theirs. Total systemic absorption of Evo and Ventolin CFC is similar

while it is higher for Evo+VOL. Silkstone and colleagues showed that Ventolin CFC attached to VOL improved lung deposition by a factor of 2.3 compared with the MDI alone. The results in this thesis show this increase in lung deposition with Evo+VOL to be by a factor of 2.9 compared to Evo alone. This increase is much larger than a factor of 1.2 reported by Hindle and Chrystyn (1994) for Ventolin CFC attached to VOL. These researchers reported USAL0.5 for Ventolin CFC alone and with VOL of 2.8% and 3.4% of the nominal dose. The USAL0.5 values as % nominal dose reported in this thesis for Evo alone are similar while Evo+VOL value is greater than Ventolin CFC attached to VOL by a factor of 2.4.

The three spacers significantly reduced the systemic availability of the dose; VOL, AERO and ABLE spacers retained 37.3%, 44.8% and 42.9% of the dispensed dose. This dose retention in spacers is reflective of their volumes and similar to those reported in this thesis for *in-vitro* studies (Table 6.2.2 & Table 6.2.13; Section 6.2.7.2). The dose remained in VOL found in the present study is lower than 54.7% and 40.6% reported respectively by Hindle and Chrystyn (1994) and Silkstone et al. (2002a) for Ventolin CFC attached to VOL.

6.3.5.1 *In-vitro in-vivo* correlation trends

FPD was similar between Evo alone and when attached with VOL and ABLE while with AERO it was significantly higher. This trend was however not translated into similar pattern of relative lung deposition (USAL0.5) in both legs of study (Table 6.3.10). The USAL0.5 of Evo alone was ~40% of Evo+SP. On the other hand, a broadly similar trend in USAL0.5 was observed with Evo+SP and mimicked their respective FPD.

Total systemic availability (USAL24NC) showed a similar trend that reflected on both *in-vitro* TDD into ACI and *in-vivo* total dose available for inhalation. Interestingly, in Part 2 Study with charcoal blockade, USAL24C amounts of Evo alone and Evo+SP approximated respectively to IP and SP+IP depositions thereby providing an estimate of salbutamol amounts which may have been swallowed and prevented from GI absorption. The ratios of FPD to USAL24C were 2.63, 1.13, 1.04 and 1.19 for Evo, Evo+VOL, Evo+AERO and Evo+ABLE, respectively. This reinforces that with charcoal blockade of GI absorption, total systemic bioavailability of salbutamol from these treatment methods was from the dose deposited in the lungs. This is reflected in

corresponding similar ratios of USAL0.5C to USAL24C of 0.18, 0.21, 0.21 and 0.23 for these treatment methods.

In-vitro and *in-vivo* relationship was more convincing within spacer treatment methods (Table 6.3.10). The trend in amounts of FPD (Evo+VOL > Evo+ABLE > Evo+AERO) and relative lung deposition (USAL0.5NC) was broadly similar (Evo+VOL > Evo+AERO ≥ Evo+ABLE) in the 1st leg of study given that mean USAL0.5NC from the latter two spacers differed by 0.4 µg only. However, USAL0.5C in the 2nd leg (with charcoal blockade) showed the same trend as that of FPD. Total delivered dose into cascade impactor (TDD (ACI)) and dose available for inhalation (TDD (NC)) in the 1st leg of study translated into the total systemic bioavailability (USAL24NC) and this trend was same as that of FPD. Deposition in IP (throat) and impactor plates (S0toF) also reflected on USAL24PreNC, USAL24PostNC and USAL24NC with the same FPD trend. It is interesting to note that FPM (stages 3, 4 & 5 deposition) followed the same trend of FPD while the trend with EPM (stages 6, 7 & filter deposition) and CPM (stages 0, 1 & 2 deposition) was different (Table 6.2.4). Given that S0toF mimicked FPD trend, it can be concluded that individual stage group may not reflect on *in-vivo* bioavailability. This is because CPM and EPM form smaller proportions of S0toF and their comparative effects on predicting *in-vivo* bioavailability may not be significant. These findings therefore reveal that *in-vitro* FPD may be a determining factor in predicting the *in-vivo* trends between spacers that are attached to Ventolin Evohaler.

Regulatory limits for the bioequivalence of formulations are that the 90% confidence limits should be between 0.80–1.25 for C_{max} and AUC (EMA, 2009). Applying these limits to the results of current study show that relative lung delivery of Ventolin Evohaler (Evo) alone was not *in-vivo* equivalent to any of the three spacers attached to it. However, total systemic delivery was *in-vivo* equivalent between Evo Vs Evo+VOL only. Nevertheless, it has been suggested that when comparing relative potencies of inhaled products these limits should be between 0.67 and 1.50 (Parameswaran, 1999). Application of these limits to the urinary salbutamol excretions in the first 30-min post-dose (USAL0.5) suggested that there was a trend for the relative lung deposition and systemic delivery of salbutamol from a Ventolin Evohaler to be similar when it was attached to Volumatic, AeroChamber Plus and Able spacer. This cautious conclusion is made due to the small number of volunteers studied (n=13); much larger numbers of subjects may need to be studied to make firmer conclusions. This comment would apply for all studies that have been shown to suggest comparability between inhaled products.

Most of the studies that were included in a meta-analysis comparing different inhalation methods also used a low number of subjects (Brocklebank et al., 2001), and were designed to show equivalence. Nevertheless, EMA (2010) recommends a minimum number of 12 evaluable subjects for any bioequivalence study. The urinary salbutamol pharmacokinetic method has been shown to be more sensitive to detect a difference in relative lung deposition than the methacholine challenge method recommended by Regulatory Authorities (Tomlinson et al., 2003). Furthermore, healthy volunteers participated in the current study and it may be that those with asthma may have different airway deposition. However, it has been shown that the only difference between volunteers and those with asthma is that lung deposition is related to airway calibre (Lipworth and Clark, 1997).

The larger relative bioavailability to the body (described by the USAL24 amounts) for the MDI method compared with that of the spacers was due to the larger emitted dose. Also, a larger proportion of the emitted dose will have been swallowed compared with when the MDI was attached to a spacer. The similar values for the 24h urinary excretion for the three spacers suggested that the amounts swallowed following inhalation of these methods was similar.

When a dose is discharged into a spacer, impaction of the particles onto its walls will increase as the size of the spacer decreases. This is because the velocity effects of the emitted plume will be greater. This is confirmed by the smaller emitted dose from the AeroChamber Plus (135 mL internal capacity) and the Able spacer (143 mL) compared with the Volumatic (820 mL). Also, despite the minimal delay between dose discharge into the spacer and inhalation, the larger volume of the Volumatic would result in more evaporation of the aerosolized dose. This contributed to a smaller particle size. These all combined to provide a dose that was emitted from the Volumatic that had a higher fine particle dose and smaller MMAD compared with the AeroChamber Plus and the Able spacer. These *in-vitro* parameters translated to the observed small (but insignificant) differences in the relative lung and systemic bioavailability of the three spacers. This observation provided further evidence of *in-vitro* and *in-vivo* correlations in line with previous suggestions (Silkstone et al., 2002c; Barry and O'Callaghan, 2003; de Matas et al., 2008). However, when this comparison was extended to the MDI alone with the spacers the link was not so clear. For example, the fine particle dose and MMAD of the MDI alone and the Volumatic were very similar yet the relative lung deposition was not. This highlights the value of inhaling from a static cloud, which occurs when using a

spacer, and suggests that comparisons that attempt to find a link between *in-vitro* and *in-vivo* data should consider the inhalation method and technique used. The higher emitted dose for the MDI compared with that of the spacers did translate into more drug being delivered to the systemic circulation (via the pulmonary and the gastrointestinal routes, with the latter predominating for the MDI).

The greater relative lung bioavailability of salbutamol for the MDI attached to a spacer compared with the MDI alone was consistent with previous reports of the corresponding CFC formulation attached to a spacer (Newman et al., 1991; Silkstone et al., 2002a). In contrast, Lipworth and Clark (1998) have shown that when using Airomir MDI, the relative lung deposition was greater when attached to a Volumatic compared with the AeroChamber, and that the latter was similar to the MDI used alone. This Airomir study did not present any *in-vitro* data to help understand the results and 12 doses were inhaled for each study dose. The effect of multiple dosing, each separated by 30 seconds, on the aerodynamics of the emitted dose was not addressed. The formulation of Airomir is different from Ventolin Evohaler, the main difference being that Airomir contains ethanol and so the emitted dose is slower and the aerosol is warmer than that of Ventolin Evohaler (Gabrio et al., 1999; Hautmann et al., 2013; Kunda et al., 2017). The difference in relative lung deposition of Airomir with large and small spacers (Lipworth and Clark, 1998) compared with contrasting results of the current study with Ventolin Evohaler pinpoint that each MDI product needs to be evaluated with different spacers before claims of interchangeability are made.

6.3.6 Conclusion

The results of this study highlight that a Ventolin Evohaler could be used with a Volumatic large volume spacer, an AeroChamber Plus or Able spacer without any difference in the relative lung and systemic delivery. This suggests that during routine use there should be no difference in the relative efficacy and safety if one of these spacers is substituted for the other when used with the Ventolin Evohaler. These results cannot be extrapolated to other inhalers and thus each formulation needs to be evaluated before a general claim of interchangeability can be made.

7 Chapter 7: *In-Vitro* and *In-Vivo* Equivalence of Airomir Without and With AeroChamber Plus

7.1 Overview

Salbutamol MDI is widely prescribed. Many patients use their MDIs correctly, and many do not, or cannot. Patients who cannot master inhaler technique will benefit from using a spacer with the MDI which makes inhaler easier to use.

Spacer use with an MDI can reduce problems relating to mismatch of actuation–inhalation coordination. A spacer slows the delivery of medication and can hold the discharged aerosol for 2-3 seconds, thereby easing coordination problems and allowing time for the patient to inhale slowly. *In-vitro* studies suggests that a spacer can reduce the impact of poorly coordinated inhalation manoeuvres (Barry and O'Callaghan, 1997; Lavorini and Fontana, 2009; Nikander et al., 2014) and even if this is completely mistimed (Foss and Keppel, 1999). Its use may also potentially improve the lung deposition of aerosolised medication as compared to MDI alone. Using gamma scintigraphy, Roller et al. (2007) have shown that use of a spacer with an MDI can result in favourable lung deposition, regardless of whether the patient performs a recommended breathing manoeuvre (slow inhalation followed by breath hold) or inhales in tidal breaths.

There is a lack of studies that use clinically relevant doses of Airomir alone and when used with a spacer. AeroChamber Plus is the recommended spacer for use with Airomir (Teva, 2016; PIL). Hence, the *in-vitro* and *in-vivo* performance of the two treatment methods was assessed and reported in this chapter in separate sections.

7.2 *In-Vitro* Equivalence of Airomir Without and With AeroChamber Plus-Aerodynamic Particle size Characterisation

The objectives of this study are to:

d) determine APSD of Airomir without and with AeroChamber Plus using ACI
e) investigate *in-vitro* equivalence between Airomir alone and with AeroChamber Plus.

7.2.1 Materials and Methods

7.2.1.1 Materials and Equipments

See Chapter 3 (Section 3.3.1.1).

7.2.1.2 Test MDI

Airomir™ (Airo).

7.2.1.3 Test Spacer

Aerochmaber Plus™ (AERO) (Figure 3.3.1).

7.2.2 Study Design

Protocols 3.3.1 and 3.3.2 (Sections 3.3.2.3 & 3.3.2.4) describe pharmacopoeia compliant study design of APSD investigations (BP, 2005; USP28-NF23, 2005; Ph. Eur., 2011) (see Chapter 3). In short, one puff of primed Airomir was discharged into ACI operated at a flow rate of 28.3 L/min for 8.5 seconds to allow 4 L of air to pass through it. The second puff was similarly discharged after 30 seconds. This procedure was repeated with AeroChamber Plus. The two treatment methods were chosen at random. AERO was pre-treated with lukewarm soapy water and drip dried after a water rinse (Section 2.4). Salbutamol was recovered from the MDI device, spacer and ACI components and stages, and quantified using a validated HPLC method (Chapter 4).

7.2.3 Deposition Profiles, CQAs and Data Analysis

The data for critical performance metrics, APSD profiles and spacer deposition were analysed as per pharmacopoeial requirements and regulatory guidelines, and described in Chapter 3 Methodology (Section 3.3.3).

7.2.4 APSD and ACI Stage Grouping

See Chapter 3 (Section 3.3.4).

7.2.5 Statistical Analysis

See Chapter 3 (Section 3.3.5).

7.2.6 Results: *In-Vitro* Equivalence of Airomir Without and With AeroChamber Plus

The mean amount (n=5) of salbutamol deposited on Airomir device, AERO and ACI are shown in Table 7.2.1. Their individual run data is provided in Appendices 5.2.3.2 and 7.2.6.1. The mass balance and TED of both treatment methods was, respectively, within 3% (RSD ≤1.8) and 25% (RSD ≤2.5) of labelled metered dose. TDD (ex-spacer) was also consistent and precise (RSD ≤3.2). Hence, APSD results of Airomir alone and with AERO are valid, accurate and precise (Christopher et al., 2003).

Figure 7.2.1 and Figure 7.2.2 respectively show deposition profiles of complete APSD and cumulative particle size for Airomir alone and with AERO. Figure 7.2.3 and Figure 7.2.4 show comparative deposition of their CQAs and stage groups, respectively.

Summaries of dose delivery metrics and their percentages are given in Table 7.2.2 and Table 7.2.3. FPD, stage group and aerodynamic characteristics and their percentages are summarised in Table 7.2.4 to Table 7.2.7. Data on dose delivery efficiencies of the two treatment methods is shown in Table 7.2.8. *In-vitro* equivalence assessment and statistical comparisons of CQAs are provided in Table 7.2.9 to Table 7.2.11.

TED (ex-actuator) of Airo Vs Airo+AERO was *in-vitro* equivalent albeit with a statistically significant difference (Table 7.2.2 & Table 7.2.9; Figure 7.2.3). TDD was neither statistically similar nor *in-vitro* equivalent as TDD (=TED) of Airomir was significantly more than that of Airo+AERO.

IP deposition of Airomir alone and with AERO was significantly different and *in-vitro* inequivalent (Table 7.2.2 & Table 7.2.11; Figure 7.2.3). These respectively constituted ~40% and ≤ 3% of their TED (Table 7.2.3). Also, the combined AERO+IP deposition was slightly more than that of IP deposition of Airomir alone. These depositions were 42% and 40% of TED, respectively, and were *in-vitro* equivalent despite being

statistically significantly different. IP+CPM deposition (non-respirable fraction) with Airomir alone was 5.5 times more than that of Airo+AERO (Table 7.2.2) and represented 3.5-fold higher proportion of TDD (Table 7.2.3). However, SP+IP+CPM deposition (non-respirable fraction) of Airo+AERO was ~3 µg more than IP+CPM deposition of Airomir alone (ratio 1.04).

S0toF deposition was similar and *in-vitro* equivalent between the two treatment methods (Table 7.2.2 & Table 7.2.9; Figure 7.2.3). Also, S0toF as %TED was *in-vitro* equivalent albeit having a marginal statistical difference (Table 7.2.3). Nevertheless, S0toF as %TDD was both statistically significantly different and *in-vitro* inequivalent.

Table 7.2.1. APSD of Airomir alone and with AeroChamber Plus.

Identity	**Airomir**			**Airo+AERO**		
	µg	SD	RSD	µg	SD	RSD
MDI Canister Valve	19.6	2.7	13.8	14.3	1.4	9.5
MDI Actuator	21.0	1.9	9.1	19.7	1.1	5.6
Spacer	-	-	-	70.8	2.6	3.7
ACI Throat	69.7	2.1	3.0	4.9	0.5	10.9
ACI S-0	3.5	0.8	24.3	2.1	0.2	9.7
ACI S-1	4.0	1.1	27.5	1.6	0.3	18.1
ACI S-2	5.8	1.4	23.7	6.5	0.8	12.7
ACI S-3	25.1	3.4	13.5	19.7	2.3	11.9
ACI S-4	35.7	1.8	4.9	40.0	3.1	7.8
ACI S-5	21.0	1.4	6.8	23.4	3.2	13.8
ACI S-6	5.2	0.5	8.7	7.5	1.4	18.7
ACI S-7	2.0	0.0	1.5	2.2	0.3	13.3
ACI Filter	2.3	0.4	17.8	2.0	0.3	15.4
Total Recovery (μg)	214.9	3.4	1.6	214.7	4.1	1.9
% Recovery[a]	107.5	1.7	1.6	107.3	2.1	1.9
Mass Balance[b] (μg)	195.3	3.5	1.8	200.3	3.4	1.7
% Recovery	97.7	1.8	1.8	100.2	1.7	1.7
TED[c] (μg)	174.3	1.7	1.0	180.6	4.4	2.4
% TED	87.2	0.8	1.0	90.3	2.2	2.4
TDD[d] (μg)				109.8	3.4	3.1
% TDD				54.9	1.7	3.1

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 μg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).
c = TED (Total Emitted Dose Ex-Actuator); Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve and Actuator (mouth piece).
d = TDD (Total Delivered Dose Ex-Spacer); Recovery calculated with respect to Nominal Dose (ND) and excludes deposition on Canister Valve, Actuator (mouth piece) and spacer.

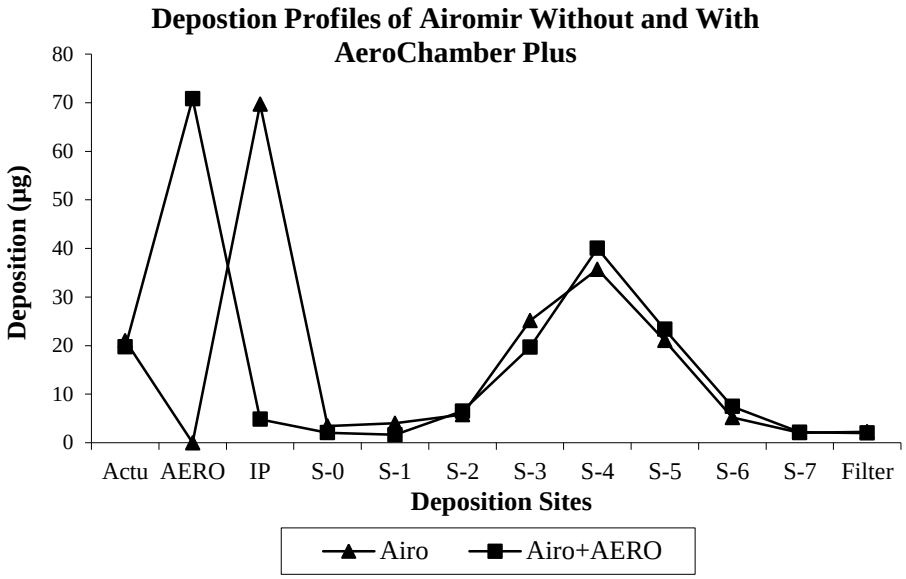


Figure 7.2.1. Complete mean APSD profiles of Airomir alone and with AeroChamber Plus.

Actu = Actuator; AERO = AeroChamber Plus; S = Stage of ACI

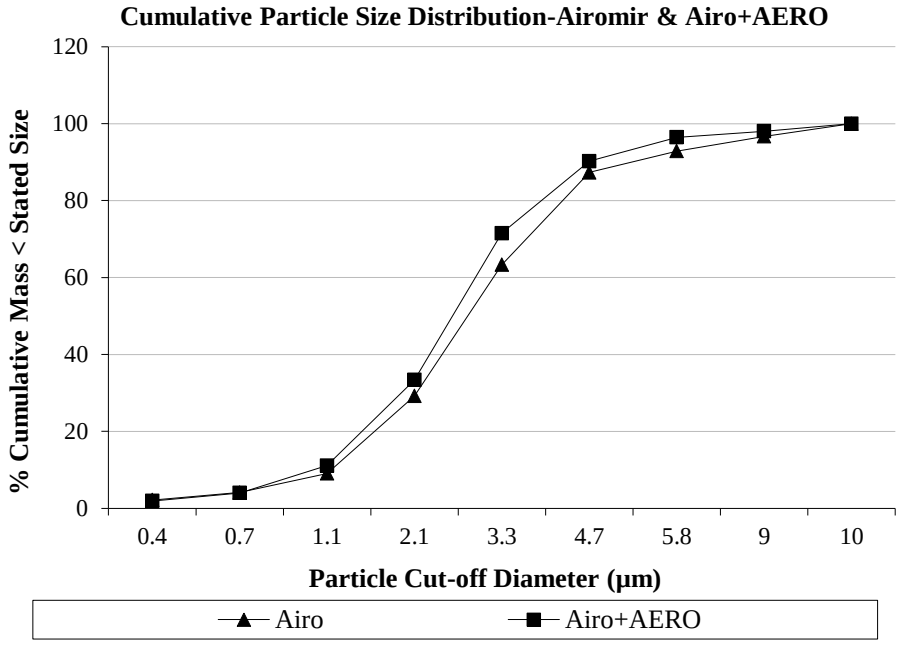


Figure 7.2.2. Mean percent cumulative particle size deposition profiles of Airomir alone and with AeroChamber Plus.

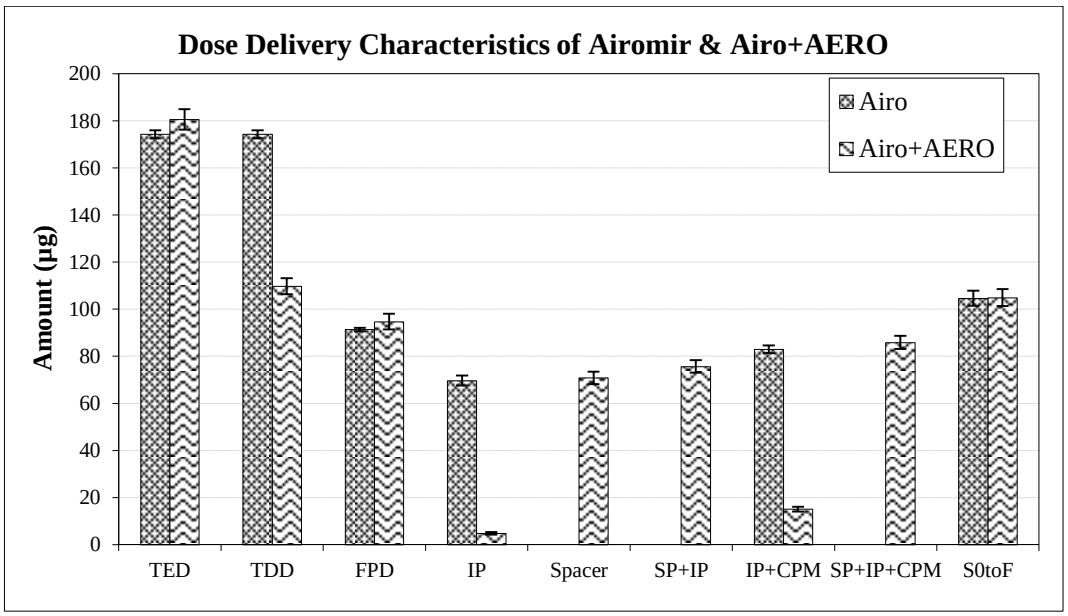


Figure 7.2.3. Dose delivery characteristics of Airomir alone and with AeroChamber Plus.

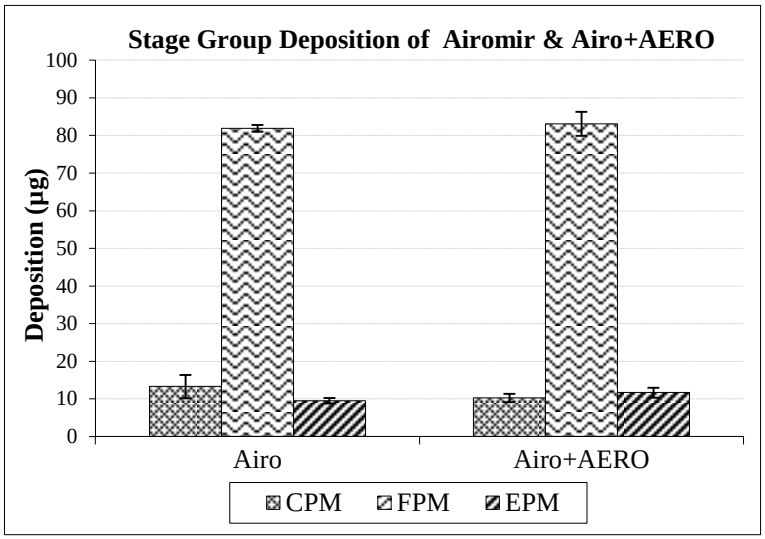


Figure 7.2.4. Stage group deposition of Airomir alone and with AeroChamber Plus.

FPD and FPM were slightly more for Airo+ AERO than those of Airomir alone and were statistically similar and *in-vitro* equivalent (Table 7.2.4, Table 7.2.9 & Table 7.2.11; Figure 7.2.3 and Figure 7.2.4). However, these CQAs constituted 86% and 76% of TDD of Airo+AERO, respectively, as compared to 52% and 47% of the MDI alone; these differences being >1.6-fold between the two treatment methods (Table 7.2.6). The fraction of FPD in TDD was therefore statistically different and *in-vitro* inequivalent (Table 7.2.9). Nevertheless, FPD as %TED (ex-actuator) was statistically similar and *in-vitro* equivalent and highlights the consistency in MDI performance.

CPM was *in-vitro* inequivalent despite being statistically similar between Airomir and Airo+AERO (Table 7.2.4 to Table 7.2.7 & Table 7.2.11; Figure 7.2.4). On the other hand, EPM was neither *in-vitro* equivalent nor statistically similar between them.

More proportion of dose was delivered to ACI as FPD (lung deposition) when Airomir was attached to AERO than without it (Table 7.2.8). FPD/IP ratio of Airo+AERO was higher than Airomir alone. However, FPD/AERO+IP ratio of Airo+AERO was lower as compared to FPD/IP ratio of Airomir alone. Similar trend was observed when FPD was compared with IP+CPM (representing oropharyngeal deposition).

MMAD of Airomir was significantly larger than that of Airo+AERO and was not *in-vitro* equivalent (Table 7.2.4 & Table 7.2.10). GSD was however statistically similar and *in-vitro* equivalent between them.

Summary of Results

TED (ex-actuator), FPD, FPF (%TED), FPM and GSD were *in-vitro* equivalent between Airomir and Airo+AERO. Moreover, FPD, FPM and GSD were also statistically similar. MMAD and GSD were smaller with Airo+AERO as compared to Airomir alone. About 39% TED (ex-actuator) was retained in AERO.

Table 7.2.2. Dose delivery and deposition in ACI of Airomir alone and with AeroChamber Plus.

Treatment Method	TED		TDD		SP		IP		SP+IP		IP+CPM		SP+IP+CPM		S0toF	
	(µg)	SD	(µg)	SD	(µg)	SD	(µg)	SD	(µg)	SD	(µg)	SD	(µg)	SD	(µg)	SD
Airomir	174.33	1.69	174.33	1.69	-	-	69.68	2.10	-	-	82.94	1.66	-	-	104.65	3.24
Airo+AERO	180.61	4.38	109.78	3.43	70.82	2.63	4.85	0.53	75.67	2.62	15.07	0.97	85.89	2.77	104.94	3.58

Table 7.2.3. Dose delivery and deposition in ACI as %TED and %TDD of Airomir alone and with AeroChamber Plus.

Treatment Method	TDD_ED		SP_ED		IP_ED		SP+IP_ED		IP+CPM_ED		SP+IP+CPM_ED		S0toF_ED		IP_DD		IP+CPM_DD		S0toF_DD	
	%TED	SD	%TED	SD	%TED	SD	%TED	SD	%TED	SD	%TED	SD	%TED	SD	%TDD	SD	%TDD	SD	%TDD	SD
Airomir	100.0	-	-	-	39.98	1.4	-	-	47.6	0.57	-	-	60.0	1.4	39.98	1.4	47.6	0.6	60.0	1.4
Airo+AERO	60.8	4.5	39.2	1.1	2.7	0.3	41.9	1.29	8.4	0.54	47.6	1.2	58.1	1.2	4.42	0.5	13.7	0.9	95.6	0.5

ED = TED; DD = TDD

Table 7.2.4. FPD, Stage groups, MMAD and GSD of Airomir alone and with AeroChamber Plus.

Treatment Method	FPD		FPM		EPM		CPM		MMAD		GSD	
	(µg)	SD	(µg)	SD	(µg)	SD	(µg)	SD	µm	SD		SD
Airomir	91.38	0.75	81.87	0.89	9.51	0.72	13.27	3.08	2.77	0.13	1.60	0.06
Airo+AERO	94.72	3.36	83.07	3.18	11.65	1.28	10.22	1.11	2.56	0.06	1.59	0.05

Table 7.2.5. FPD and stage groups as %TED of Airomir alone and with AeroChamber Plus.

Treatment Method	FPF (%)		FPM_TED		EPM_ED		CPM_ED	
	%TED	SD	%TED	SD	%TED	SD	%TED	SD
Airomir	52.42	0.57	46.96	0.51	5.46	0.43	7.60	1.71
Airo+AERO	52.44	1.20	45.99	1.27	6.45	0.67	5.66	0.60

ED = TED

Table 7.2.6. FPD and stage groups as %TDD of Airomir alone and with AeroChamber Plus.

Treatment Method	FPF (%)		FPM_DD		EPM_DD		CPM_DD	
	%TDD	SD	%TDD	SD	%TDD	SD	%TDD	SD
Airomir	52.42	0.57	46.96	0.51	5.46	0.43	7.60	1.71
Airo+AERO	86.27	0.90	75.65	0.72	10.62	1.16	9.31	0.99

DD = TDD

Table 7.2.7. FPD and stage groups as %S0toF of Airomir alone and with AeroChamber Plus.

Treatment Method	FPD_S0toF		FPM_S0toF		EPM_S0toF		CPM_S0toF	
	% S0toF	SD	% S0toF	SD	% S0toF	SD	% S0toF	SD
Airomir	87.38	2.59	78.28	2.22	9.10	0.80	12.62	2.59
Airo+AERO	90.26	1.01	79.16	0.91	11.11	1.21	9.74	1.01

S0toF = Impactor mass (Deposition on stages S0-S7+F)

Table 7.2.8. FPD and S0toF delivery efficiency of Airomir alone and with AeroChamber Plus.

Treatment Method	FPD / SP		FPD / IP		FPD / SP+IP		FPD / IP+CPM		FPD / SP+IP+CPM		S0toF / IP		S0toF / SP+IP	
	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD
Airomir	-	-	1.31	0.04	-	-	1.10	0.03	-	-	1.50	0.09	-	-
Airo+AERO	1.34	0.07	19.74	2.55	1.25	0.06	6.31	0.48	1.10	0.05	21.88	2.88	1.39	0.07

Table 7.2.9. *In-Vitro* Equivalence and Statistical Significance of CQAs of Airomir alone and with AeroChamber Plus.

Airomir Vs Airo+AERO Parameter	Mean Ratio	90% CI		*p value*	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
		LL	UL				LL	UL		
TED	1.04	1.01	1.06	0.017	Yes	3.14	0.72	5.56	0.017	No
TDD	0.63	0.61	0.65	<0.0001	No	-32.27	-34.24	-30.30	<0.0001	No
FPD	1.04	1.01	1.07	0.062	Yes	1.67	-0.11	3.44	0.062	Yes
FPF (%TED)	1.00	0.98	1.02	0.989	Yes	0.02	-1.35	1.39	0.977	Yes
FPF (%TDD)	1.65	1.63	1.67	<0.0001	No	33.85	32.75	34.95	<0.0001	No
S0toF	1.00	0.97	1.04	0.901	Yes	0.14	-2.34	2.63	0.897	Yes
S0toF (%TED)	0.97	0.94	0.99	0.050	Yes	-1.93	-3.84	-0.01	0.049	No*
S0toF (%TDD)	1.59	1.56	1.63	<0.0001	No	35.55	33.99	37.12	<0.0001	No

*Marginally significant; CQA = Critical Quality Attribute; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 7.2.10. *In-Vitro* Equivalence and Statistical Significance of MMAD and GSD of Airomir alone and with AeroChamber Plus.

Airomir Vs Airo+AERO Parameter	**Mean Ratio**	**90% CI**		***p value***	***In-Vitro Equivalence* (0.85-1.18)**	**Mean Difference**	**95% CI**		***p* value**	**Statistical Similarity**
		LL	**UL**				**LL**	**UL**		
MMAD (µm)	0.87	0.82	0.92	0.001	No	-0.21	-0.35	-0.07	0.009	No
GSD	0.92	0.88	0.95	0.003	Yes	-0.01	-0.09	0.07	0.839	Yes

Table 7.2.11. *In-Vitro* Equivalence and Statistical Significance of IP and Stage Groups of Airomir alone and with AeroChamber Plus.

Airomir Vs Airo+AERO Parameter	**Mean Ratio**	**90% CI**		***p value***	***In-Vitro Equivalence* (0.85-1.18)**	**Mean Difference (µg)**	**95% CI**		***p* value**	**Statistical Similarity**
		LL	**UL**				**LL**	**UL**		
SP+IP*	1.09	1.05	1.13	0.004	Yes	3.00	1.26	4.73	0.004	No
IP (Throat)	0.07	0.06	0.08	<0.0001	No	-32.41	-33.53	-31.30	<0.0001	No
Group 1 (CPM)	0.79	0.63	0.98	0.080	No	-1.52	-3.21	0.16	0.071	Yes
Group 2 (FPM)	1.01	0.98	1.05	0.451	Yes	0.60	-1.10	2.30	0.439	Yes
Group 3 (EPM)	1.22	1.09	1.37	0.011	No	1.07	0.31	1.83	0.012	No

*(Spacer + Throat) (MDI SP+IP = IP)

7.2.7 Discussion: *In-Vitro* Equivalence Studies of Airomir Without and With AeroChamber Plus

AeroChamber Plus (AERO) is named spacer for use with Airomir as and when required (Teva, 2014). TED of Airo+AERO was 3.6% (3 µg per puff) more than that of Airomir alone. Although this difference between the two treatment methods was statistically significant, yet both were *in-vitro* equivalent. However, with Airo+AERO, 61% of TED was delivered into ACI while 39% of TED was retained within AERO. Hence, there was a significant difference in TDD between them which was also manifest in their *in-vitro* inequivalence.

Mitchell et al. (1999) and Ross and Gabrio (1999) have reported TDD of 64.2 µg and 74.4 µg (calculated from FPF) when Airomir was used with AeroChamber (a predecessor of AERO). These TDDs are higher than that of current study by 9.3 µg (ratio 1.17) and 19.5 µg (ratio 1.36), respectively. Further, the TDD of Ross and Gabrio was 10.2 µg greater than that of Mitchell et al. (ratio 1.16). These investigators however used 20 and 5 puffs of Airomir, respectively.

The likelihood of electrostatic forces in retaining Airomir dose in AERO is not being ruled out. However, since AERO was pre-treated with mild detergent to dissipate such forces (Table 2.4.2), it is assumed that the presence of these electrostatic forces was minimal (Dewsbury et al., 1996; Kwok et al., 2006) and may have existed at a similar level irrespective of the material used in their manufacture (Chuffart et al., 2001; Coppolo et al., 2006). Further, detergent treatment does not eliminate all charge on the spacer and a minimum ground charge is inherently present (Dewsbury et al, 1996; Kwok et al., 2006; Prabhakaran et al., 2012).

It is reported that the emitted salbutamol sulphate particles acquire charge due to frictions with the device components and actuator surfaces (Noakes, 2004; Kwoke et al., 2006; Mitchell and Nagel, 2007). In the presence of a minimum residual charge on the spacer internal surfaces, it is likely that some electrostatic interaction would take place and removes and/or deflects aerosol particles from the mainstream. It has been shown that the aerosol cloud of salbutamol suspension MDI contains large particles, droplets and multiplets (droplets containing multiple drug particles) immediately after emission (Sheth et al., 2015) and that the presence of ethanol slows down their

evaporation and dispersion due to its relatively slower evaporating speed than the HFA propellants (Barry and O'Calaghan, 1997; Ross and Gabrio, 1999; Stein and Myrdal, 2006). It has also been shown that the diameter of Proventil HFA (US equivalent of Airomir) plume was larger in the immediate vicinity of actuator (Hautmann et al., 2013; Johnson et al., 2016; Kunda et al., 2017). Citing Hinds (1982), Coppolo et al. (2006) reported that the charge acquired by field, and also partly by diffusion, increases as a strong function of particle size within the range of 0.4–10 μm aerodynamic diameter. Coppolo et al. elaborated that this phenomenon may increase electrical mobility of larger particles, and therefore likelihood of their electrostatic capture on the interior surfaces of the spacer will be greater. Hence, when large numbers of puffs are used sequentially and are fired separately into the spacer, the emitted particles of the initial puffs would interact with the residual charge on the un-primed spacer surface more than those released by the later puffs; the magnitude of this particle interaction would be particle size dependent. This would therefore influence the total amount of drug leaving the spacer which may alter lung deposition (Mitchell and Nagel, 2007).

Priming the interior surfaces of a spacer with several puffs of the medication has been found to increase whole lung deposition (Kenyon et al., 1998; Rau et al., 2006). Accordingly, studies employing numerous doses further reduce the electrostatic charge on a spacer (Terzano, 2001). Wilkes et al. (2001) used 5 puffs of salbutamol MDI to prime the internal surfaces of spacers to reduce the electrostatic charge while Berg et al. (1998) used 15 actuations for inhaled corticosteroids (ICS). Australian Asthma Handbook (v1.2) suggests that in hospitals and emergency departments, a new spacer can be primed using multiple (at least 10) puffs of salbutamol. The GINA guidelines (2017) recommend using at least 20 puffs into a new unwashed chamber before delivery of a rescue dose. Given that Airomir contains oleic acid, which is a surfactant, the effect of several doses may be more pronounced. Since clinically relevant 2 puffs of Airomir were used in the current study, this antistatic priming effect may be lacking and explains the differences in TDD found with the studies by Mitchell et al. (1999) and Ross and Gabrio (1999). Regression analysis of the number of puffs and TDD of these two studies along with the current study revealed a linear relationship between these metrics (R^2=0.888; y = 0.953x + 55.92), highlighting that TDD increased with increasing the number of Airomir puffs. Hence, it is clear that the differences in TDD between these studies were due to the higher number of puffs used by these investigators. On another

note, the TDD results of the current study are more reflective on the actual use scenario of AERO by the patients.

AERO significantly reduced IP deposition by 93% (65 µg). This difference in IP deposition between the two treatment methods was 37% of TED (ex-actuator). Although 14 times more non-respirable dose was deposited in IP with Airomir alone than Airo+AERO, yet combined AERO+IP deposition of Airo+AERO was 6 µg (ratio 1.09) more than IP deposition of the MDI alone (Table 7.2.2). Higher combined AERO+IP deposition was also noted with Ventolin Evohaler (3.4 µg; ratio 1.08) and Salamol (4.3 µg; ratio 1.11) when compared with their respective IP depositions of MDI alone (Table 6.2.2 & Table 7.2.2, respectively). These findings confirm that spacers remove the ballistic portion of the dispensed dose that would otherwise impact on the throat and oral cavity (Table 2.5.2). This may have clinical implications for systemic effects that may be caused by oropharyngeal deposition and consequent GI absorption when the MDI is used alone.

Further, non-respirable dose of Airo+AERO (AERO+IP+CPM) was 1.04 times more than that of Airomir alone (IP+CPM). Similar trend was observed between the two treatment methods when respirable dose (FPD) was compared with non-respirable dose (IP+CPM and AERO+IP+CPM) (Table 7.2.8). The results show that AERO retained more of the non-respirable dose while produced lesser CPM than Airomir alone. Additionally, AERO reduced significant proportion of TED without affecting APSD profile of Airomir. This would reflect on the efficacy of the two treatment methods.

Impactor mass of Airo+AERO as %TDD was 96% which was 1.6-fold more than that of 60% with Airomir alone (Table 7.2.3). This is also reflected in their respective ratios of S0toF to IP deposition of 21.9 and 1.5, indicating that salbutamol delivery to S0toF was 15-fold more efficient with Airo+AERO than the MDI alone (Table 7.2.8). This finding from derived data implies that drug delivery to the human respiratory tract (HRT) would be better with the spacer (Airo+AERO) treatment method. However, comparison of the ratios of S0toF to AERO+IP for Airo+AERO (1.39) to that of S0toF to IP of Airomir alone suggests otherwise and indicates that both treatment methods will have similar dose delivery to the HRT. This is consistent with findings that statistically similar and *in-vitro* equivalent impactor mass (S0toF) was recovered with both treatment methods. Also, comparison of lung to throat (FPD/IP & FPD/AERO+IP) and respirable to non-respirable (FPD/IP+CPM & FPD/AERO+IP+CPM) fractions of the

two treatment methods reveal similar respective trends between them. Again, this would imply a better FPD delivery with Airo+AERO which was, however, only 1.7 µg (per actuation) more than that of MDI alone (ratio 1.04). Interestingly, FPD of the two treatment methods was statistically similar and *in-vitro* equivalent (Table 7.2.9). In contrast, Mitchell et al. (1999) and Ross and Gabrio (1999) reported significantly higher FPD when Airomir was used with AeroChamber than the MDI alone in their studies; this difference was 23 µg (ratio 1.60) and 14.7 µg (ratio 1.30), respectively. These investigators however used 5 and 20 puffs of Airomir, respectively. Again, a linear correlation was found when TDD was compared with FPD of these studies with the current study (R^2=0.837; y = 0.866x + 2.060). As discussed earlier, these differences are related to the number of puffs used which showed a ratcheting effect on spacer dose delivery output and consequently on FPD. Hence, the results of studies which use many puffs can be misleading and can have clinical ramifications.

Mitchell et al. (1999) and Ross and Gabrio (1999) reported FPD of 62 µg and 64.5 µg, respectively with the spacer treatment method which are significantly higher than that found with the current study having respective ratios of 1.31 and 1.36. , their FPD of the MDI alone are 38.7 µg and 49.8 µg having ratios of 0.85 and 1.09, respectively, to the FPD of the current study which lies between this range. Further, FPD of Airomir reported by Barry and O'Calaghan (1997), Dubus et al. (2001) and Johnson et al. (2016) are 37.2 µg (10 Puffs), 54.1 µg (5 puffs) and 40.1 µg (6 puffs) and their respective ratios to the current FPD are 0.81, 1.18 (< 5.8 µm) and 0.88. The analyses of these results further substantiate that use of several Airomir puffs with the detergent treated AeroChamber proportionally increased FPD in the *in-vitro* studies. It is known that spacers improve FPD delivery (Newman and Newhouse, 1996; Newman, 2004); however, this improvement should be shown in the clinically relevant dose. The results of current study confirm FPD improvement in clinically relevant dose of Airomir when used with AERO.

The results of the current study show that FPF as %TED was similar and *in-vitro* equivalent between Airomir alone and when used with AERO. AERO retained most of large particle mass and emitted dose leaving it contained ~86% of FPF as %TDD. However, even with these advantages, it is known that use of a spacer cannot entirely obviate the need for coordination and a delay can reduce the amount of drug available to inhalation (Barry and O'Callaghan, 1997; Rau, 2006; Mitchell and Nagel, 2007; Slator et al., 2014; Liu et al., 2017). The analyses of actual and derived data

indicate that delivery of salbutamol to the lungs from Airo+AERO may be more efficient, however, not better than that from Airomir alone. Patient preference and compliance studies have revealed that they do not always adhere to using spacer with the MDI medication (Laube et al., 2011; Levy et al., 2016). The findings of the current study suggest that patients would still have equal benefits from Airomir alone because the FPD is similar and provided that they are able to inhale correctly. Further, Airomir may be a better choice for patients who could master inhalation technique and can tolerate systemic effects. Nevertheless, this assessment applies only to AERO.

FPM (3–5 µm) deposition represents the mass which is more likely to deposit in bronchi and bronchioles (Pritchard, 2001). FPM was *in-vitro* equivalent and statistically similar between Airomir alone and Airo+AERO. Although FPM of Airo+AERO was only 1.2 µg more than that of Airomir alone, these constituted 77% and 47% of TDD, respectively. However, their contribution to impactor mass (S0toF) was about similar at 79% and 78%, respectively (Table 7.2.7). Significantly greater and *in-vitro* inequivalent EPM was delivered with Airo+AERO than Airomir alone and formed 11% and 9% of their respective S0toF. This is consistent with the benefits of using a spacer which allows the emitted aerosol more space and time to form finer particles (Lavorini and Fontana, 2009; Laube et al., 2011). Higher EPM may also contribute to systemic effects. Nevertheless, EPM differs by only 2 µg between the two treatment methods and therefore may not be of clinical significance despite being 18% in magnitude. CPM (Group 1 stages, > 5 µm) was however statistically similar though not *in-vitro* equivalent between the two treatment methods. CPM of Airomir alone was 3 µg more than that of Airo+AERO and constituted 13% and 10% of their respective S0toF. This is expected, however, the small difference is perhaps surprising even though the magnitude of this difference is paradoxically 23%. Although salbutamol dose in particle size of 6 µm (S2 deposition) has shown bronchodilation (Usmani et al., 2003), whether this small difference in CPM amounts can have any clinical significance would be difficult to identify. Nonetheless, the use of derived data may lead to inherently incorrect conclusions and decisions (also see Section 6.2.7.5). Since derived data at times has a tendency to inflate the results, in particular for smaller amounts, regulatory authorities have always asked for the raw data (actual results).

MMAD highlights the midpoint of APSD while GSD shows the dispersion around this MMAD. Significantly higher MMAD was obtained with Airomir alone as compared to Airo+AERO (Table 7.2.4 & Table 7.2.10). Airomir contains co-solvent ethanol and

surfactant oleic acid, and therefore produces slow and longer lasting puff (Barry and O'Calaghan, 1997; Ross and Gabrio, 1999; Hautmann et al., 2013; Johnson et al., 2016). It can be hypothesized that when a puff of Airomir is discharged into AERO, the propellant and co-solvent have enough space and time to evaporate and form fine particles before being carried away into ACI with airflow with a higher proportion of finer particles. This is evident from the relatively higher EPM and lower CPM for Airo+AERO than Airomir alone (Table 7.2.4); their respective EPM and CPM ratios were 1.23 and 0.77 while FPD/CPM ratios being 9.3 and 6.9. It is therefore likely that higher proportion of CPM found with Airomir alone may have additionally contributed to larger MMAD and GSD. Nevertheless, these MDI performance metrics lie in the desired size range required for relieving bronchospasm. Given that FPD and FPM of the two treatment methods were *in-vitro* equivalent and statistically similar, these differences in MMAD and GSD are unlikely to affect clinical outcome.

7.2.8 Conclusions: *In-Vitro* Equivalence of Airomir Without and With AERO

1. The data suggests that Airomir can be used without AERO, provided that patients are skilful in inhalation manoeuvres.
2. AERO has shown to be a compatible add-on device with Airomir; depicting similar impactor mass profiles and generating *in-vitro* equivalent FPD while significantly reducing IP deposition. This could be beneficial to those patients with press-and-breathe coordination difficulties and those hypersensitive to known side effects of salbutamol.
3. Finer MMAD and GSD were obtained with Airo+AERO than Airomir alone. Higher CPM found with MDI alone may have impacted MMAD size.
4. Raw quantitative data should be used to make comparative assessment of MDI performance metrics. Derived data needs to be assessed within the overall context.
5. Findings of this study apply only to Airomir and AERO and cannot be extended to other MDIs and spacers.

7.3 *In-Vivo* Equivalence of Airomir Without and With AeroChamber Plus-Urinary Pharmacokinetic Studies

MDIs require coordination of press and breathe manoeuvres by patient to ensure effective drug delivery to the lungs (Crompton, 1982; Chapman et al., 1993; Crompton et al., 2006). Poor inhalation technique can decrease pulmonary deposition (Newman et al., 1991b), increase oropharyngeal deposition and reduce therapeutic effect (Lindgren et al., 1987; Chapman et al., 1993). The use of spacer can minimise these phenomena (Newman, 2004; Lavorini and Fontana, 2009).

Accordingly, the objectives of this study are to investigate the effects of AeroChamber Plus on the relative lung and total systemic bioavailability of Airomir in healthy subjects using urinary pharmacokinetic method (Hindle and Chrystyn, 1992). A charcoal blockade study is also conducted to estimate salbutamol lung deposition from Airomir alone and when used with AeroChamber Plus.

7.3.1 Study Design

The Study plan is elaborated in Sections 5.3.1 (Chapter 5) and 6.3.1 (Chapter 6).

In short, this study comprised of two parts, each part with two sub-sets involving two methods of salbutamol inhalation, vis-à-vis: Airomir alone (Airo) and with AeroChamber Plus (Airo+AERO). In Part 1 Study, on separate study day (one week apart) trained healthy subjects inhaled two separate 100 µg doses of salbutamol from one of these treatment methods selected randomly. Each dose discharged from primed Airo or into pre-washed AERO (see Protocols 3.3.1 & 3.3.2 (Sections 3.3.2.3 & 3.3.2.4; Chapter 3)) was inhaled using a slow vital capacity inhalation manoeuvre (Hindle et al., 1993). Each single dose discharged into AERO was inhaled within the first second of discharge into the spacer. All subjects provided their blank urine 0.5h pre-dosing. Urine samples were then collected 0.5h after the start of each study dose (USAL0.5) and thereafter subjects pooled all their urine over the next 24h into a container (USAL24). In Part 2 Study, each subject repeated this study with the concurrent administration of activated charcoal by swallowing 100 mL of charcoal slurry immediately before and after two inhalations.

The pH and volume of each sample was recorded and samples were stored at –20°C before analysis. The pH values of the urine samples were all below pH 7, hence there was no variability due to passive tubular reabsorption (Hindle and Chrystyn, 1992).

After inhalation of the two study doses each spacer was rinsed with water to collect the residual dose.

7.3.2 Sample Analysis

Aqueous and urine samples were assayed for their salbutamol content using validated HPLC methods described in Chapter 4.

7.3.3 Statistical Analysis

See Section 3.4.7 (Chapter 3).

7.3.4 Results: *In-Vivo* Equivalence of Airomir Without and With AeroChamber Plus

Demographic characteristics of volunteers are given in Table 5.3.1 & Appendix 5.3.4.1. Summaries of USAL0.5 and USAL24 amounts post-dose (without and with charcoal blockade) are provided in Table 7.3.1 and Figure 7.3.1 to Figure 7.3.5. These table and figures also show salbutamol as active (USAL24Pre), active and sulphate conjugated (USAL24Post) and metabolised (USALMET) moieties excreted during 0.5h to 24h period. Comparative salbutamol urinary recovery profiles obtained post-inhalation without and with charcoal blockade are shown in Figure 7.3.6. Mean salbutamol urinary excretions as % nominal, % delivered and % recovered dose are given in Table 7.3.2 to Table 7.3.4. Urinary excretion data of individual subjects for Airomir is given in Appendices 5.3.4.3 and 5.3.4.6. Appendices 7.3.4.1 and 7.4.3.2 provide individual data for Airo+AERO for both legs of the study. Table 7.3.5 provides data on *in-vivo* equivalence and comparative bioavailability of the two inhaled salbutamol treatment methods for both parts of the study.

In Part 1 Study, relative lung bioavailability (USAL0.5NC) of Airomir attached to AERO was significantly greater than the MDI alone and was not *in-vivo* equivalent (Table 7.3.1 & Table 7.3.5). The amount of USAL0.5NC of Airo+AERO was more than twice that of Airo alone. However, total systemic bioavailability (USAL24NC) between them was *in-vivo* equivalent as per EMA (2009) criteria and statistically similar. The excretion of active salbutamol (USAL24PreNC) and its metabolites (USALMETnc) were not *in-vivo* equivalent while total salbutamol (USAL24PostNC) met this criterion. Further, USAL24PreNC and USAL24PostNC were *in-vivo* equivalent as per limits

suggested by Parameswaran (1999). Nonetheless, bioavailability of only USAL24PostNC was statistically similar.

In Part 2 Study with charcoal blockade, none of these PK parameters were *in-vivo* equivalent between Airo and Airo+AERO as per EMA (2009) criteria (Table 7.3.1 & Table 7.3.5). However, their total systemic bioavailability (and also USAL24PostC) was *in-vivo* equivalent as per Parameswaran (1999) criteria. Moreover, the bioavailability of only USAL24PostC and USALMETc was statistically similar between them. The USAL0.5C amount of Airo+AERO was greater than Airo alone by a factor of 2.2.

The charcoal block effect is compared in Table 7.3.6 and Figure 7.3.7. Data reveals significant differences between these parameters except for USAL0.5 which was statistically bioequivalent between the two legs of the study.

Table 7.3.1. Mean salbutamol excretion in urine post-inhalation from Airomir without and with AeroChamber Plus.

Treatment Method (n = 13)	**USAL0.5**		**USAL24†**		**USAL24Pre**		**USAL24Post**		**USALMET**	
	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD
Part 1 Study (without charcoal blockade)										
Airomir	7.1	2.1	84.2	13.1	47.4	7.6	77.1	12.7	29.8	8.3
Airo+AERO	15.1	4.2	83.9	8.1	56.9	6.6	68.8	5.4	11.9	4.7
Part 2 Study (with charcoal blockade)										
Airomir	6.7	2.2	48.5	11.9	29.7	9.0	41.8	10.8	12.2	3.9
Airo+AERO	14.3	4.0	62.3	11.1	39.2	7.8	48.0	8.2	8.7	4.2

† TRD = USAL24; SD = Standard Deviation

Table 7.3.2. Mean salbutamol excretion in urine post-inhalation from Airomir without and with AeroChamber Plus, expressed as % of Nominal Dose.

Treatment Method (n = 13)	**USAL0.5**		**USAL24†**		**USAL24Pre**		**USAL24Post**		**USALMET**	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Airomir	3.6	1.0	42.1	6.5	23.7	3.8	38.6	6.4	14.9	4.2
Airo+AERO	7.5	2.1	42.0	4.0	28.5	3.3	34.4	2.7	6.0	2.4
Part 2 Study (with charcoal blockade)										
Airomir	3.3	1.1	24.3	5.9	14.8	4.5	20.9	5.4	6.1	1.9
Airo+AERO	7.1	2.0	31.1	5.5	19.6	3.9	24.0	4.1	4.4	2.1

† TRD = USAL24; SD = Standard Deviation

Table 7.3.3. Mean salbutamol excretion in urine post-inhalation from Airomir without and with AeroChamber Plus, expressed as % of Delivered Dose.

Treatment Method (n = 13)	USAL0.5		USAL24†		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Airomir	4.0	1.1	47.7	7.5	26.8	4.4	43.7	7.3	16.8	4.7
Airo+AERO	14.9	3.7	83.4	5.4	56.6	5.0	68.5	3.9	11.9	4.7
Part 2 Study (with charcoal blockade)										
Airomir	3.8	1.3	27.6	6.9	16.9	5.2	23.8	6.2	6.9	2.2
Airo+AERO	14.2	4.3	61.7	12.0	38.9	8.5	47.5	8.7	8.6	4.0

† TRD = USAL24; SD = Standard Deviation

Table 7.3.4. Mean salbutamol excretion in urine post-inhalation from Airomir without and with AeroChamber Plus, expressed as % of Recovered Dose.

Treatment Method (n = 13)	USAL0.5		USAL24†		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Airomir	8.5	2.3	-	-	56.5	6.2	91.5	2.3	35.0	6.4
Airo+AERO	17.8	3.7	-	-	67.8	3.8	82.2	3.7	14.4	6.0
Part 2 Study (with charcoal blockade)										
Airomir	14.0	4.0	-	-	60.5	6.2	86.0	4.0	25.5	6.9
Airo+AERO	22.8	3.7	-	-	63.0	4.6	77.2	3.7	14.3	6.9

† TRD = USAL24; SD = Standard Deviation

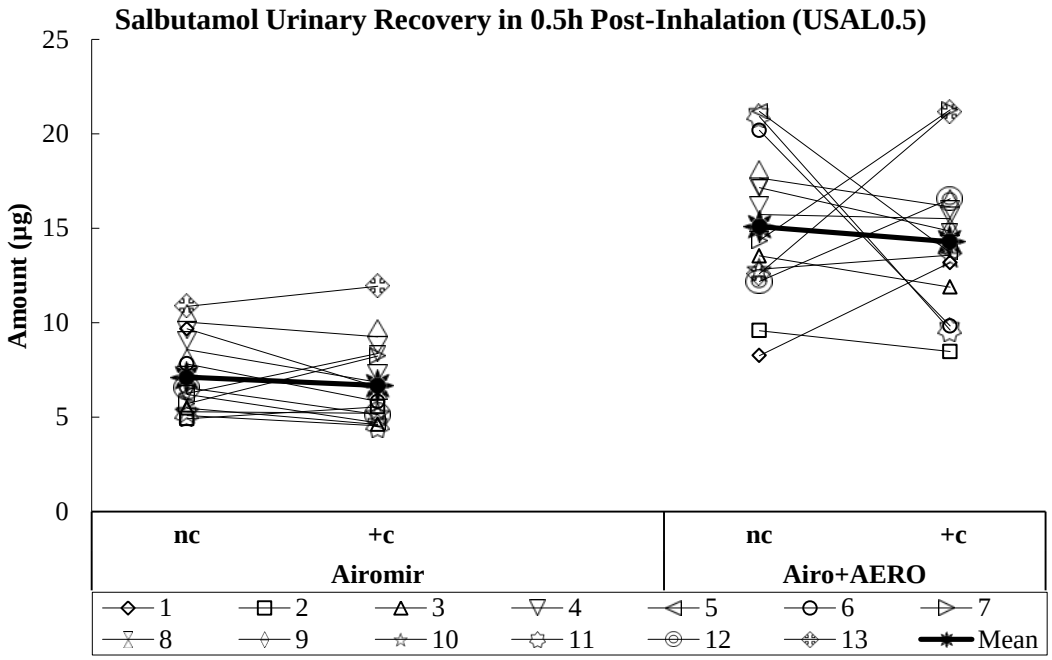


Figure 7.3.1. Comparative salbutamol urinary excretion at 0.5h post-inhalation of Airomir without and with AeroChamber Plus.

Numerals represent individual volunteers. nc = no charcoal ingestion; +c = with charcoal ingestion.

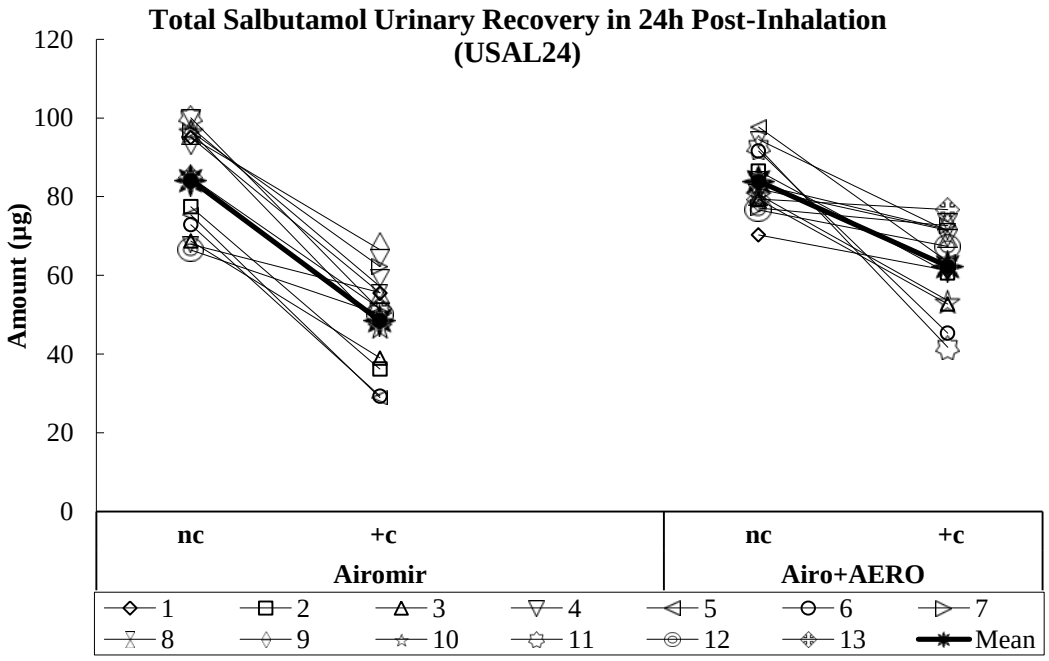


Figure 7.3.2. Comparative total salbutamol urinary excretion during 24h post-inhalation of Airomir without and with AeroChamber Plus.

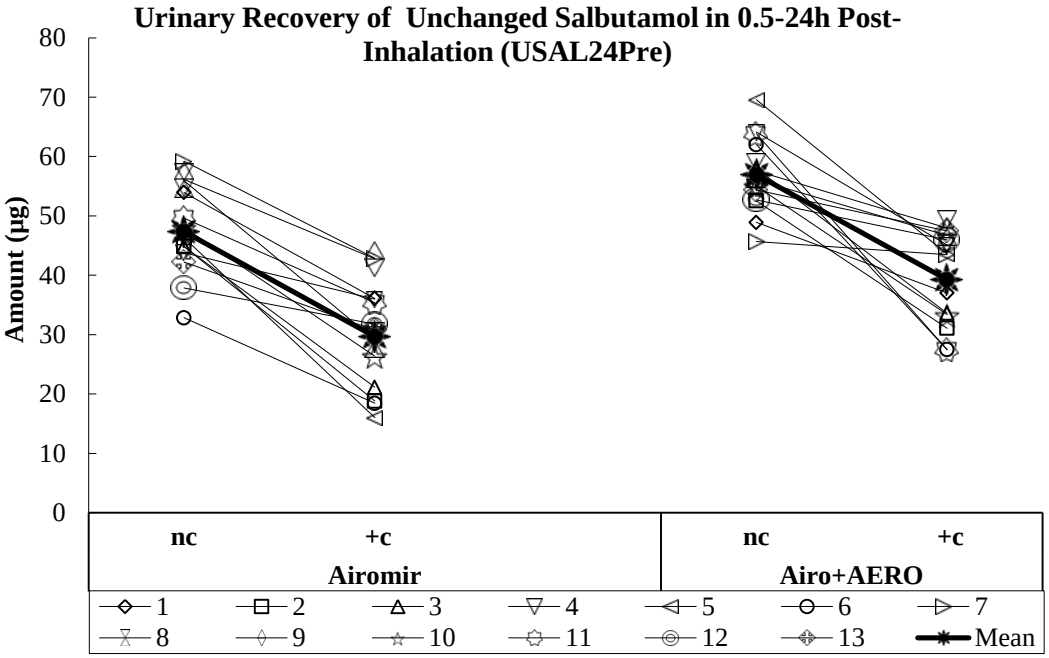


Figure 7.3.3. Comparative unchanged salbutamol urinary excretion during 0.5-24h post-inhalation of Airomir without and with AeroChamber Plus.

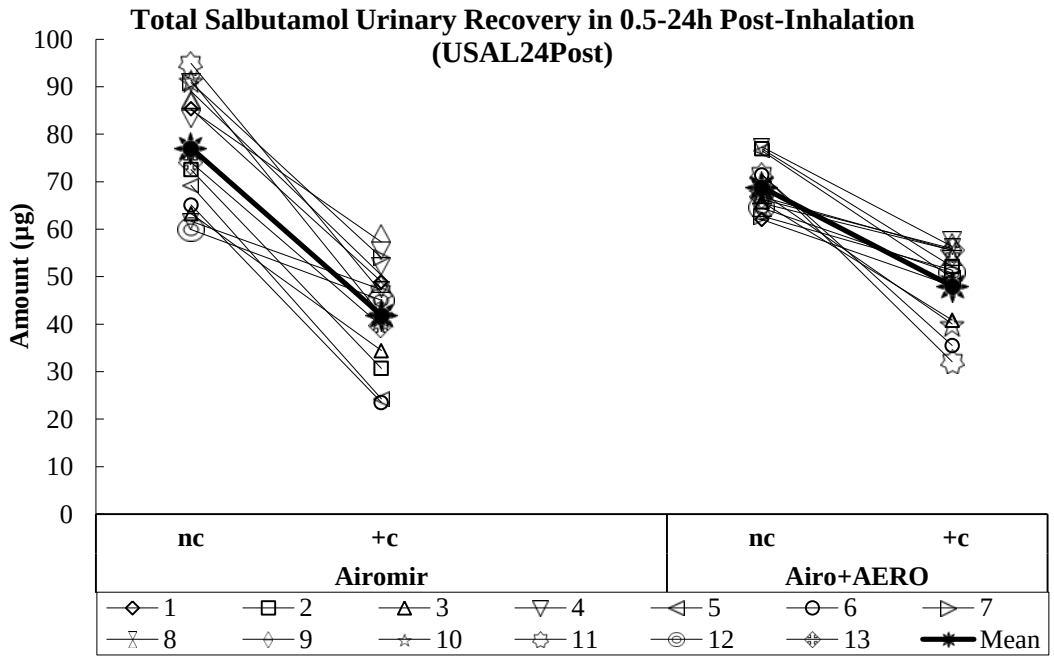


Figure 7.3.4. Comparative total salbutamol urinary excretion during 0.5-24h post-inhalation of Airomir without and with AeroChamber Plus.

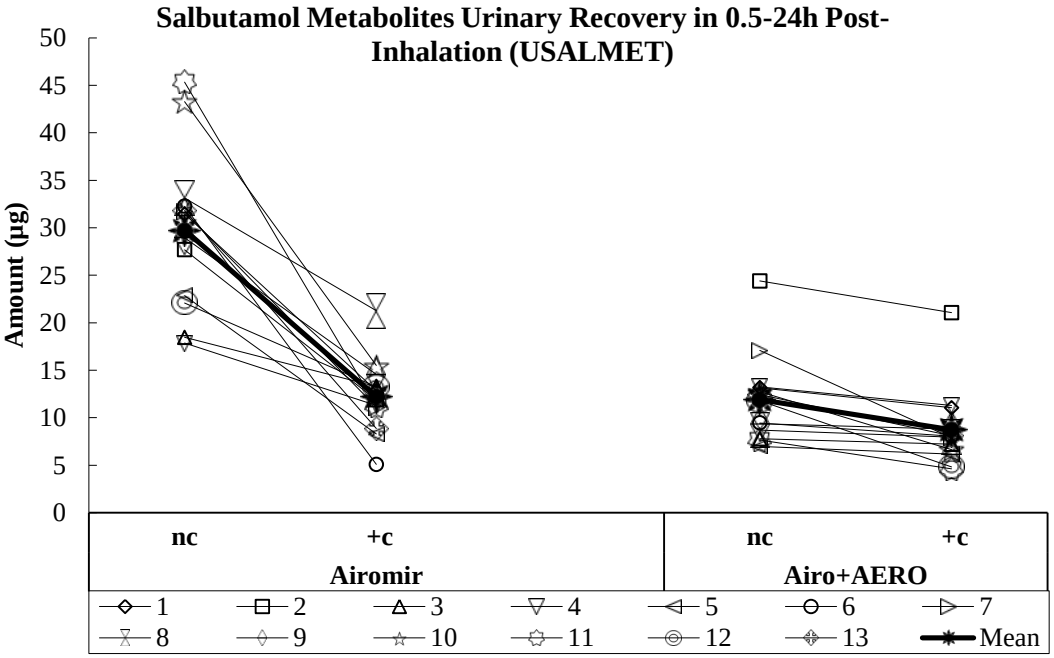


Figure 7.3.5. Comparative salbutamol metabolites urinary excretion during 0.5-24h post-inhalation of Airomir without and with AeroChamber Plus.

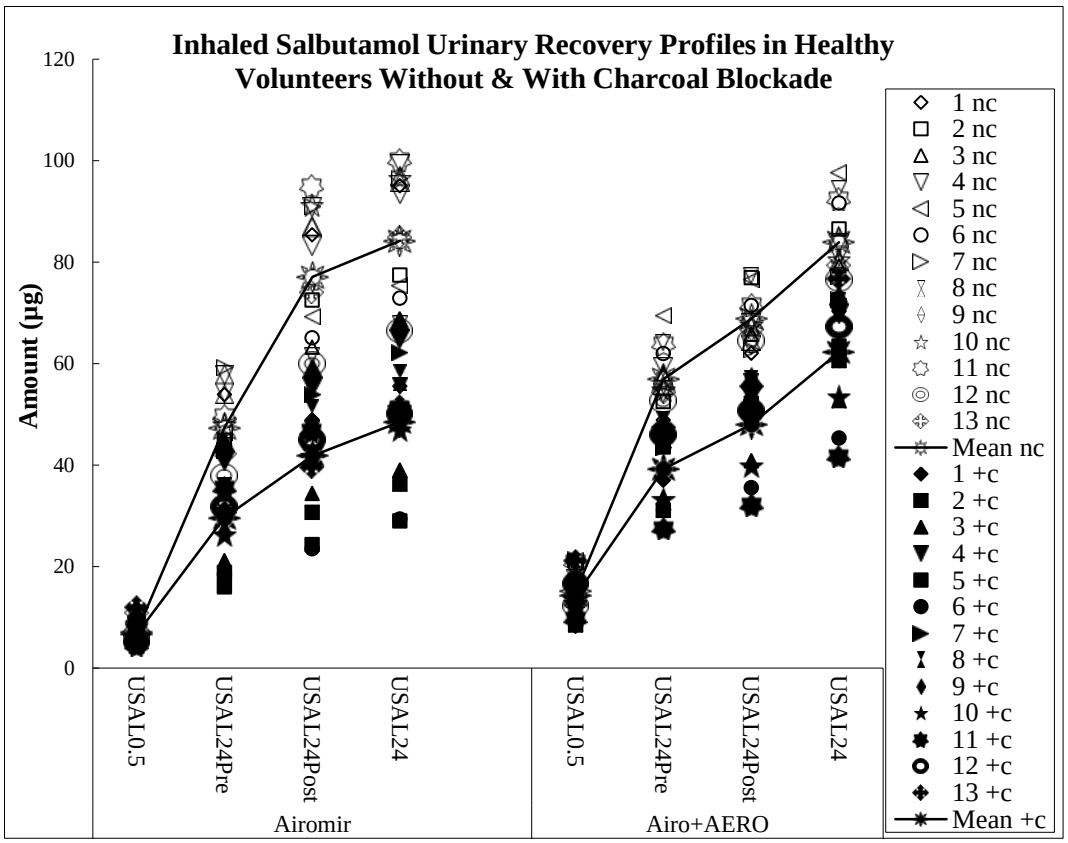


Figure 7.3.6. Comparative salbutamol urinary recovery profiles obtained post-inhalation of Airomir without and with AeroChamber Plus.

Numerals represent individual volunteers. nc = no charcoal ingestion; +c = with charcoal ingestion.

Table 7.3.5. *In-Vivo* Equivalence and Statistical Significance of Airomir without and with AeroChamber Plus.

Parameter	Mean Ratio	90% CI		*p value*	*In-Vivo Equivalence*		Mean Difference (µg)	95% CI		*p* value	Statistical Similarity
		LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
Airo+AERO Vs Airomir: Part 1 Study (without charcoal blockade)											
USAL0.5NC	2.12	1.72	2.61	<0.0001	No	No	7.98	5.04	10.91	<0.0001	No
USAL24NC	1.00	0.91	1.11	0.950	Yes	Yes	-0.29	-10.72	10.14	0.953	Yes
USAL24PreNC	1.21	1.08	1.36	0.013	No	Yes	9.59	2.47	16.71	0.013	No
USAL24PostNC	0.90	0.81	1.00	0.098	Yes	Yes	-8.27	-17.66	1.12	0.079	Yes
USALMETnc	0.39	0.31	0.49	<0.0001	No	No	-17.86	-23.74	-11.97	<0.0001	No
Airo+AERO Vs Airomir: Part 2 Study (with charcoal blockade)											
USAL0.5C	2.16:	1.92	2.43	<0.0001	No	No	7.61	5.91	9.31	<0.0001	No
USAL24C	1.30	1.16	1.47	0.002	No	Yes	13.76	7.15	20.37	0.001	No
USAL24PreC	1.36	1.17	1.59	0.004	No	No	9.60	4.08	15.11	0.003	No
USAL24PostC	1.17	1.02	1.35	0.071	No	Yes	6.15	-0.44	12.74	0.065	Yes
USALMETc	0.70	0.54	0.90	0.027	No	No	-3.45	-6.93	0.03	0.052	Yes

[a] EMA, 2009; [b] Parameswaran, 1999; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 7.3.6. Statistical comparison of salbutamol post-inhalation urinary excretion between Part 1 and Part 2 studies of Airomir without and with AeroChamber Plus.

Parameter [nc Vs (+c)	**Treatment**	**Mean paired Difference**	**95% CI**		***t value***	***p value***	**Statistical Similarity**
			LL	**UL**			
USAL0.5	Airomir	0.44	-0.56	1.43	0.957	0.358	Yes
	Airo+AERO	0.80	-2.94	4.54	0.467	0.649	Yes
USAL24	Airomir	35.71	28.67	42.74	11.058	<0.0001	No
	Airo+AERO	21.66	12.30	31.01	5.045	<0.0001	No
USAL24Pre	Airomir	17.71	13.16	22.25	8.483	<0.0001	No
	Airo+AERO	17.70	11.11	24.28	5.856	<0.0001	No
USAL24Post	Airomir	35.27	28.49	42.05	11.332	<0.0001	No
	Airo+AERO	20.86	14.83	26.89	7.536	<0.0001	No
USALMET	Airomir	17.57	12.29	22.84	7.257	<0.0001	No
	Airo+AERO	3.16	1.49	4.83	4.118	0.001	No

CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

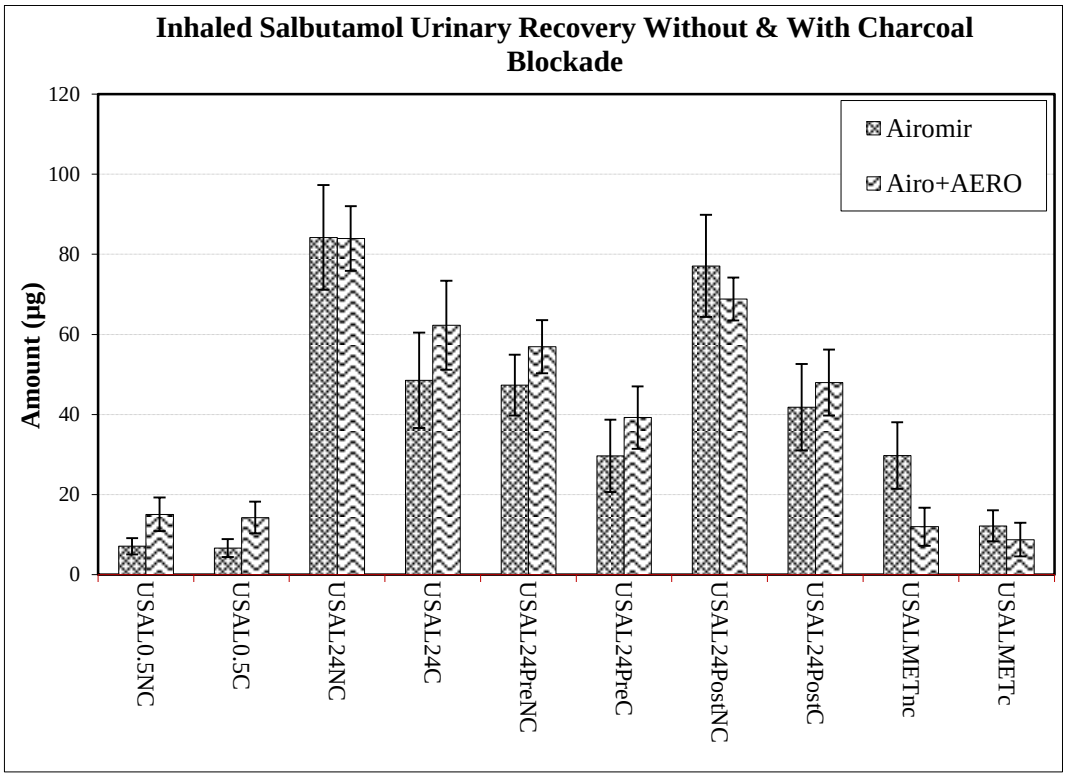


Figure 7.3.7. Comparison of salbutamol post-inhalation urinary excretion of Airomir without and with AeroChamber Plus between Part 1 and Part 2 studies.

7.3.5 Discussion: *In-Vivo* Equivalence of Airomir Without and With AeroChamber Plus

Part 1 Study revealed that AeroChamber Plus used with Airomir significantly increased relative lung deposition (USAL0.5NC); this increase being ≥2-fold (Table 7.3.1). The two treatment methods therefore did not meet the EMA (2009) *in-vivo* equivalence criterion for USAL0.5NC (Table 7.3.5). These findings may have implications for efficacy.

On the other hand, total systemic bioavailability (USAL24NC) was statistically similar and bioequivalent, indicating that the total systemic delivery from either Airomir alone or with AERO was not affected by the treatment method. This suggests that both treatment methods may have similar pattern of systemic effects. This is perhaps surprising given that AERO retained a large proportion of the dispensed dose (40% ND; 44% TED) that would otherwise additionally contribute to systemic effects via GI absorption. However, significantly greater active salbutamol was excreted in the first 0.5h and during 0.5-24h (USAL24PreNC) post-inhalation of Airo+AERO, suggesting that the two treatment methods may have similar systemic effects. This is further supported by the findings that USAL24PreNC formed 29% and 68% of ND and TRD, respectively, for Airo+AERO as compared to corresponding 24% and 57% for Airomir alone (Table 7.3.2 & Table 7.3.4). Nevertheless, it has been shown that the side effects from cumulative doses of 100 to 4000 µg of salbutamol MDI were well tolerated and infrequent in asthmatics (Lipworth et al., 1988b). Hence, it is unlikely that a total systemic exposure of 84 µg over 24 hours (USAL24NC) may be of concern.

In Part 2 Study with charcoal blockade, the increase in Airomir dose delivery to the lungs with AERO as compared to the MDI alone was similar to that in the first leg of the study, i.e., without charcoal blockade. This reaffirms that the urinary excretion of inhaled salbutamol in the first 0.5h was mainly derived from the proportion of the dose deposited in the lungs. This is reflected in the significant differences in total systemic bioavailability (USAL24C) of the two treatment methods (Table 7.3.1 and Table 7.3.5).

The ratio of USAL24C to USAL24NC suggests that ~58% and ~74% of total systemic bioavailability of Airomir and Airo+AERO, respectively, was from their lung deposition (Table 7.3.1). For Airomir, USAL24C was ~28% of the total delivered dose as compared to ~48% of USAL24NC suggesting that ~20% of the delivered dose may have been swallowed (Table 7.3.3). For Airo+AERO, these figures were

respectively ≤62%, ≥83% and ~22%. Similarity of swallowed proportion of inhaled salbutamol from the two treatment methods suggest that charcoal block was consistent. This also shows that when Airomir is used with AERO, relatively lesser amount of the dispensed dose is swallowed. Further, the ratio of the active salbutamol excretion over 0.5-24h post-inhalation (USAL24PreC Vs USAL24PreNC) as %TDD shows that ~63% and 68% was derived from the lung deposition of the two respective treatment methods (Table 7.3.3). Also, USAL24PreNC and USAL24PreC, respectively, constituted ~57% and ~61% of the total systemic bioavailability for Airomir alone and ~68% and ~63% for Airo+AERO (Table 7.3.4). These results suggest that higher lung deposition of Airomir with AERO in 0.5h post inhalation resulted in higher active salbutamol over 0.5-24h post-inhalation as is evident from the charcoal blockade of the GI absorption.The metabolised salbutamol (USALMETc) recovery in Part 2 Study was statistically similar between the two treatment methods (Table 7.3.5). However, USALMETnc was 2.5-fold more with Airomir alone than Airo+AERO in Part 1 Study (Table 7.3.1). This finding reflects on higher proportion of swallowed salbutamol that undergoes consequent enterohepatic metabolism and is consistent with the role of spacers in reducing the swallowed proportion of the inhaled salbutamol. Interestingly, the proportion of metabolised salbutamol in total systemic bioavailability (USAL24) was the same at 14% for Airo+AERO in both legs of the study (Table 7.3.4) and highlights consistency in Airomir dose delivery when used with AERO. This finding also reflects on the similarity of swallowed portion of salbutamol from the two treatment methods as mentioned above.

The current study shows that lung deposition was increased when AERO was attached to Airo as compared to the MDI alone. This finding reaffirms that spacer improve lung depositions while reducing total systemic bioavailability (Newman et al., 1991a). This is consistent with previous reports for Ventolin CFC (Hindle and Chrystyn, 1994), Ventolin Evohaler (Mazhar and Chrystyn, 2008), Intal MDI (sodium cromoglycate) (Aswania et al., 1999), Cromogen MDI (sodium cromoglycate) (Aswania and Chrystyn, 2001) and Bricanyl MDI (terbutaline) (Abdelrahim, 2009). In contrast, a study using plasma salbutamol as a surrogate marker of lung deposition found no significant difference between AeroChamber attached to Airomir and when the MDI was used alone even though plasma salbutamol peak (C_{max}) and average (C_{av}) were higher with the spacer treatment method (Lipworth and Clark, 1998). AeroChamber is the earlier

version of AeroChamber Plus and has relatively smaller volume (145 mL) than that of the latter (149 mL). These investigators, however, did not elaborate on as to how much dose remained in AeroChamber and the dose that was available for inhalation. Electrostatic charge on a static spacer can significantly reduce dose delivery of an MDI by electrostatistically pulling away emitted particles of the aerosol cloud from the mainstream (Mitchell and Nagel, 2007; Nikander et al., 2014). However, pre-washing a spacer with detergent liquid minimises this interaction (Section 2.4). Since AeroChamber was not subjected to such pre-treatment in their study, it is likely that a larger proportion of the emitted dose may have been retained in it due to electrostatic forces on the spacer walls with consequences in low dose available for inhalation. This may have masked the differences in plasma salbutamol pharmacokinetics of the two treatment methods. In a later study by the same group (Fowler et al., 2001), AeroChamber was prewashed with detergent and primed with 50 puffs. This resulted in increase in C_{max} and C_{av} which was 1.48-fold (32%) and 1.42-fold (30%) greater than those of the MDI alone. This is in line with the result of current study.

The results show that the total systemic bioavailability of Airomir alone and when used with AERO was similar and bioequivalent (Table 7.3.1Table 7.3.1 & Table 7.3.5). This finding is consistent with the results of Ventolin Evohaler used alone and when attached to AERO (Mazhar and Chrystyn, 2008; Table 6.3.9). These results apparently may look contradicting given that larger proportion of dose (88% of ND) was available for inhalation when Airomir was used alone as compared to when used with AERO (50% of ND). However, in Part 2 Study with charcoal blockade (Table 7.3.1 to Table 7.3.5), the total systemic bioavailability (USAL24C) of Airomir alone was significantly lower than that of Airo+AERO, constituting 24% and 31% of the nominal dose respectively, despite being bioequivalent as per limits suggested by Parameswaran (1999). It is therefore clear that this difference in total systemic bioavailability is due the significant and bio-inequivalent differences in their relative lung deposition (USAL0.5C). With the coadministration of activated charcoal, the GI absorption of swallowed portion of Airomir dose was prevented and consequently USAL24C originated from USAL0.5C. Since USAL0.5C of Airomir was less than half of Airo+AERO, this was reflected in its relatively lower USAL24C. Further, USAL0.5 as % of TRD reflected on the urinary excretion of active salbutamol over 0.5-24h (USAL24Pre) of the two treatment methods in both legs of the study, suggesting that higher lung deposition resulted in higher systemic bioavailability of active salbutamol. Hence, it can be concluded that

statistically similar and bioequivalent total systemic bioavailability in Part 1 Study was due to significantly greater lung deposition from Airo+AERO.

USAL24Pre in both legs of study broadly reflect on the trend (Airo+AERO > Airo) of *in-vitro* deposition on the impactor plates (S0toF) (Airo+AERO ≥ Airo), the latter mimics the dose delivery to the respiratory tract (Table 3.3.2; Figure 2.3.1). This is in line with the significantly smaller IP (throat) deposition with Airo+AERO and shows that the non-respirable fraction of the emitted dose was removed by AERO. The ratios of FPD/IP were 1.3 and 19.7 for Airo and Airo+AERO respectively and highlight the significantly greater *in-vitro* dose delivery efficiency of the latter treatment method. In Part 2 Study, USAL24C also followed this trend reconciling to the above assertion that in the presence of charcoal blockade of GI absorption, salbutamol entered systemic circulation via the lung route. This is substantiated by significantly higher *in-vitro* FPF of 52% and 86% of these treatment methods, respectively. However, this trend was marginally reversed with USAL24NC in Part 1 Study (Airo ≥ Airo+AERO). Nevertheless, both USAL24NC and S0toF were statistically similar and equivalent between the two treatment methods in their respective *in-vivo* and *in-vitro* studies.

The FPD and FPF of the dispensed dose represent the respirable amount and fraction that can reach the lungs which account for the amount excreted 0.5h post-inhalation (USAL0.5). The *in-vitro* finding that Airo+AERO was more efficient than Airo alone in delivering the FPD is reflected in higher relative lung bioavailability (USAL0.5) with the former albeit with a smaller magnitude (Table 7.2.8 & Table 7.3.1). The increase in FPD could not reach statistical significance while the increase in USAL0.5NC did achieve this level between the two treatment methods (Table 7.2.9 & Table 7.3.5). However, %FPF (%TDD) of Airo+AERO was 1.7-fold greater than that of MDI alone (Table 7.2.6) and mirrors the 2.1-fold greater USAL0.5NC (Table 7.3.1) and its fraction in the total systemic recovery (USAL24NC) (Table 7.3.4). This is consistent with the *in-vitro* higher FPD delivery efficiency with Airo+AERO (Table 7.2.8). The ratio of respirable (FPD) to non-respirable (IP+CPM) dose with Airo+AERO was 5.7-fold greater than that of MDI alone which is greater than the ratio of USAL0.5NC between the two treatment methods.

The large improvement in the relative lung deposition with Airo+AERO over Airo alone has been obtained in subjects who have been trained and demonstrated good

inhalation technique. Besides, the inhalation manoeuvre was completed within 30 seconds of an actuation. However, many patients do not inhale correctly (Molimard et al., 2003; Brennan et al., 2005; Crompton et al., 2006; Chrystyn and Price, 2009; Laube et al., 2011; Levy et al., 2013 & 2016; Aggarwal and Gogtay, 2014; Bonini and Usmani 2015; Sanchis et al., 2016), and this has been observed even after training (Hardwel et al., 2009). Liu et al. (2017) have recently reported a significant difference between *in-vitro* TED, FPD, FPF for Proventil HFA (USA equivalent of Airomir) without and with AERO. They also reported that for Proventil+AERO, these MDI performance metrics were significantly influenced by patient handling and compliance parameters mimicked by inhalation delay between actuation and inhalation, and variable inhalation flow rates. Given that relative lung bioavailability (USAL0.5) of Airomir with AERO was 2-fold greater than the MDI alone, this could compensate for the small errors in inhalation manoeuvres without adversely affecting the effective lung dose.

Gunawardena et al. (1997) compared the efficacy of 2 puffs (100 µg each) of salbutamol CFC MDI (Baker Norton, UK) administered to asthmatics using two types of spacers. They found that the lowest available salbutamol dose was 50 µg and that the dose response (measured as FEV_1, FVC & PEF) tended to be flat above 200 µg. However, Clark and Lipworth (1997) found in asthmatics that 400 µg dose represented the steep part of the dose-response curve for bronchodilatation and that dose-related systemic effects of salbutamol occurred at higher doses (>500 µg). Nevertheless, Lipworth and McDevitt (1989) reported that in normal subject airways a plateau in bronchodilatation occurs with much lower doses of inhaled salbutamol. Further, Fishwick et al. (2001) found no difference in the bronchodilating response to single doses of 50, 100, or 400 µg of salbutamol administered via Turbuhaler after either 5 or 25 min indicating that 50 µg of salbutamol produced a bronchodilation close to the maximum administration and that there was no FEV_1 dose–response relationship associated with single doses of salbutamol within this range. These researchers concluded that the inhaled dose from the inhaler devices therefore may be higher than that required by most patients with asthma. Given that Airomir is effective and safe at the recommended dosage (Dockhorn et al., 1995 & 1997; Bleecker et al., 1998; PIL), the greater relative lung bioavailability of Airo+AERO found in the current study would be beneficial and may not raise safety concerns even though pharmacodynamics study may be required to prove this.

Singh et al. (2011) using plasma profiles determined lung bioavailability and total systemic exposure of Foster (Chiesi) without and with AERO. They found increased peak plasma concentrations (C_{max}) and the $AUC_{(0,30\ min)}$ of beclomethasone dipropionate, its active metabolite beclometasone 17-monopropionate (17-BMP) and formoterol with AERO as compared to the MDI alone. The increases in the AUC(0,30 min), indicative of lung deposition, for 17-BMP and formoterol were 41% and 45% respectively, while the total systemic exposure measured as $AUC_{(0,t\ last)}$ remained the same between the two treatment methods. They also evaluated the lung deposition using the charcoal blockade without AERO and found that plasma concentrations were not influenced by charcoal ingestion, and that systemic exposure to Foster actives was from lung absorption. They reported that more than 30% of the inhaled dose of Foster was delivered to the lung using the MDI alone after ingestion of charcoal which confirmed $AUC_{(0,30\ min)}$ as an index of lung bioavailability. Although the contribution of AERO in relative lung deposition (USAL0.5) of Airomir is significantly greater than that found with Foster, the results of their study in general support the findings of the current study.

7.3.6 Conclusion

Applying bioequivalence limits suggested by Parameswaran (1999), the results of current study show that relative lung delivery of Airomir alone was not *in-vivo* equivalent to when it was attached to AeroChamber Plus. However, total systemic delivery was *in-vivo* equivalent between the two treatment methods. Since AeroChamber Plus removed a large proportion of the non-respirable dose and delivered more dose to the lungs, therefore it could be used with Airomir. These results are MDI and spacer specific and hence cannot be extrapolated to other treatment methods.

8 Chapter 8: *In-Vitro* and *In-Vivo* Equivalence of Salamol Without and With Spacer

8.1 Overview

Salamol HFA is one of the salbutamol MDIs available in the UK. Little is known about its *in-vitro* performance characteristics while *in-vivo* studies are hard to find. Further, *in-vitro* studies complemented with *in-vivo* studies of Salamol with spacers have not been reported to-date. Hence, these studies have been conducted to compare Salamol when used alone and with two spacers, vis-à-vis: Volumatic and AeroChamber Plus. Volumatic is the recommended spacer for Salamol to overcome issues of inadequate inhalation technique (Teva, 2016). AeroChamber Plus is promoted as universal spacer and therefore has been included in this study.

This chapter is structured into separate sections of *in-vitro* and *in-vivo* equivalence studies on Salamol without and with spacers.

8.2 *In-Vitro* Equivalence of Salamol Without and With Spacer-Aerodynamic Particle size Characterisation

The objectives of this study are to:

a) determine APSD of Salamol without and with spacer using ACI
b) investigate *in-vitro* equivalence between Salamol alone and with Volumatic and AeroChamber Plus.

8.2.1 Materials and Methods

8.2.1.1 Materials and Equipments

Details are provided in Chapter 3 (Section 3.3.1.1).

8.2.1.2 Test MDI

Salamol™ (Sala).

8.2.1.3 Test Spacers

Volumatic™ (VOL) and AeroChamber Plus™ (AERO) (Figure 3.3.1).

8.2.2 Study Design

Salamol MDI, spacers and ACI equipment were prepared and studies conducted as detailed in Protocols 3.3.1 and 3.3.2 (Sections 3.3.2.3 & 3.3.2.4; Chapter 3). In summary, two puffs of the primed Salamol were fired into ACI separately with a gap of 30 seconds. ACI was operated at a flow rate of 28.3 L/min for 8.5 seconds for each puff allowing 4 L of air to pass through it. Salbutamol was recovered from the MDI components, spacer and ACI assembly, and quantified by HPLC (Chapter 4).

Each of the three Salamol treatment methods (MDI alone, Sala+VOL and Sala+AERO) was selected randomly. The two spacers were pre-treated with lukewarm soapy water, rinsed with clean water and drip dried before use (Section 2.4).

8.2.3 Deposition Profiles, CQAs and Data Analysis

The details are provided in Chapter 3 (Section 3.3.3).

8.2.4 APSD and ACI Stage Grouping

These are defined in Chapter 3 (Section 3.3.4).

8.2.5 Statistical Analysis

Data analysed as described in Chapter 3 (Section 3.3.5).

8.2.6 Results: *In-Vitro* Equivalence of Salamol Without and With Spacer

The recovery of salbutamol from Salamol MDI, spacers and ACI is given in Table 8.2.1. APSD and cumulative particle size deposition profiles are shown in Figure 8.2.1 and Figure 8.2.2. Comparison of CQAs is provided in Figure 8.2.3 and Figure 8.2.4. Table 8.2.2 to Table 8.2.7 show comparative data on CQAs while Table 8.2.8 provides an insight into the dose delivery efficiency of the three treatment methods. Data for individual ACI experiments for Salamol, Sala+VOL and Sala+AERO are given in Appendices 5.2.3.3, 8.2.6.1 and 8.2.6.2, respectively.

Table 8.2.1 shows that mass balance and TED of the three treatment methods were within 5% (RSD $\leq$3.0) and 25% (RSD <3.0), respectively, of labelled metered dose. Therefore, APSD results are valid, accurate and precise (Christopher et al., 2003).

The results show that TED (ex-actuator), FPD and FPF (%TED) were *in-vitro* equivalent and statistically similar between the three Salamol treatment methods (Table 8.2.2, Table 8.2.4 to Table 8.2.6, Table 8.2.8 & Table 8.2.8). TDD of the two spacer treatment methods was also *in-vitro* equivalent and statistically similar between them. However, TDD (ex-spacer) was *in-vitro* inequivalent and statistically significantly different from the TDD (=TED ex-actuator) of the MDI. FPF as %TDD was significantly higher with VOL (32%) and AERO (40%) than Salamol alone, hence resulting in their *in-vitro* inequivalence. Nevertheless, FPF as %TDD was *in-vitro* equivalent between the two spacer treatment methods albeit having significant statistical difference.

VOL retained ~8.6% less Salamol than AERO (Table 8.2.2 & Table 8.2.3; Figure 8.2.3). The IP deposition of Salamol MDI alone was significantly more than the two spacer treatment methods and this translated into their *in-vitro* inequivalence (Table 8.2.11).

Impactor mass (S0toF) of the three treatment methods was statistically similar and *in-vitro* equivalent except that it was not *in-vitro* equivalent between the MDI and Sala+AERO (Table 8.2.9). Further, S0toF as %TED was within 5% of each other

(Table 8.2.3). However, when assessed as % TDD, S0toF was over 39% greater with the two spacers than the MDI alone.

Table 8.2.1. APSD of Salamol alone and with spacers.

Identity	Salamol			Sala+VOL			Sala+AERO		
	µg	SD	RSD	µg	SD	RSD	µg	SD	RSD
MDI Canister Valve	20.0	2.2	10.8	7.5	1.1	15.2	12.6	1.4	11.4
MDI Actuator	28.8	5.3	18.4	19.9	2.1	10.6	21.2	2.0	9.6
Spacer	-	-	-	77.7	5.7	7.3	84.3	6.9	8.1
ACI IP (Throat)	80.1	3.1	3.8	5.9	1.3	21.3	4.4	0.5	10.8
ACI S-0	2.7	0.6	22.4	2.4	0.2	10.4	1.1	0.2	21.1
ACI S-1	3.8	0.8	21.3	4.5	0.5	11.5	1.4	0.2	17.3
ACI S-2	4.1	0.5	11.9	6.7	0.8	12.6	2.7	0.5	18.9
ACI S-3	18.9	2.5	13.4	21.9	2.6	11.8	20.6	5.1	24.6
ACI S-4	35.8	4.3	12.1	32.7	2.4	7.3	34.2	2.9	8.5
ACI S-5	24.0	3.8	15.8	19.6	1.6	8.2	21.5	5.1	23.7
ACI S-6	5.8	1.0	16.9	4.9	1.5	30.6	5.1	1.5	29.9
ACI S-7	2.3	0.5	21.5	2.1	0.4	19.6	2.0	0.3	14.4
ACI Filter	2.6	0.8	31.7	2.4	0.1	3.5	2.3	0.3	13.9
Total Recovery (μg)	228.9	7.4	3.2	208.1	4.8	2.3	213.4	2.3	1.1
% Recovery[a]	114.4	3.7	3.2	104.1	2.4	2.3	106.7	1.1	1.1
Mass Balance[b] (μg)	208.8	6.2	3.0	200.7	3.7	1.8	200.7	2.4	1.2
% Recovery	104.4	3.1	3.0	100.3	1.9	1.8	100.4	1.2	1.2
TED[c] (μg)	180.0	5.2	2.9	180.7	2.3	1.3	179.6	2.8	1.6
% TED	90.0	2.6	2.9	90.4	1.1	1.3	89.8	1.4	1.6
TDD[d] (μg)	-	-	-	103.0	5.1	4.9	95.2	5.6	5.8
% TDD	-	-	-	51.5	2.5	4.9	47.6	2.8	5.8

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 μg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).
c = TED (Total Emitted Dose Ex-Actuator); Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve and Actuator (mouth piece).
d = TDD (Total Delivered Dose Ex-Spacer); Recovery calculated with respect to Nominal Dose (ND) and excludes deposition on Canister Valve, Actuator (mouth piece) and spacer.

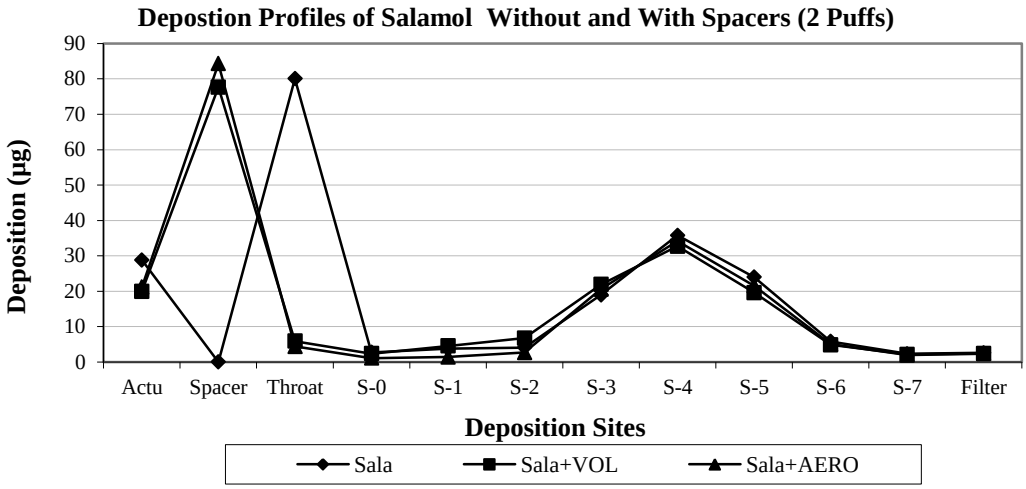


Figure 8.2.1. Complete mean APSD profiles of Salamol alone and with spacers.

Actu = Actuator; S = Stage of ACI

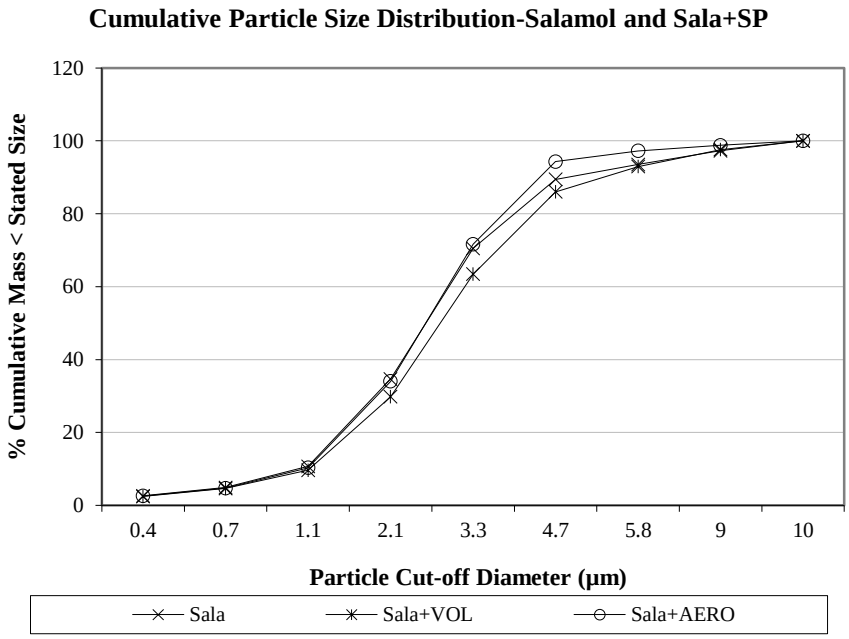


Figure 8.2.2. Mean percent cumulative particle size deposition profiles of Salamol alone and with spacers.

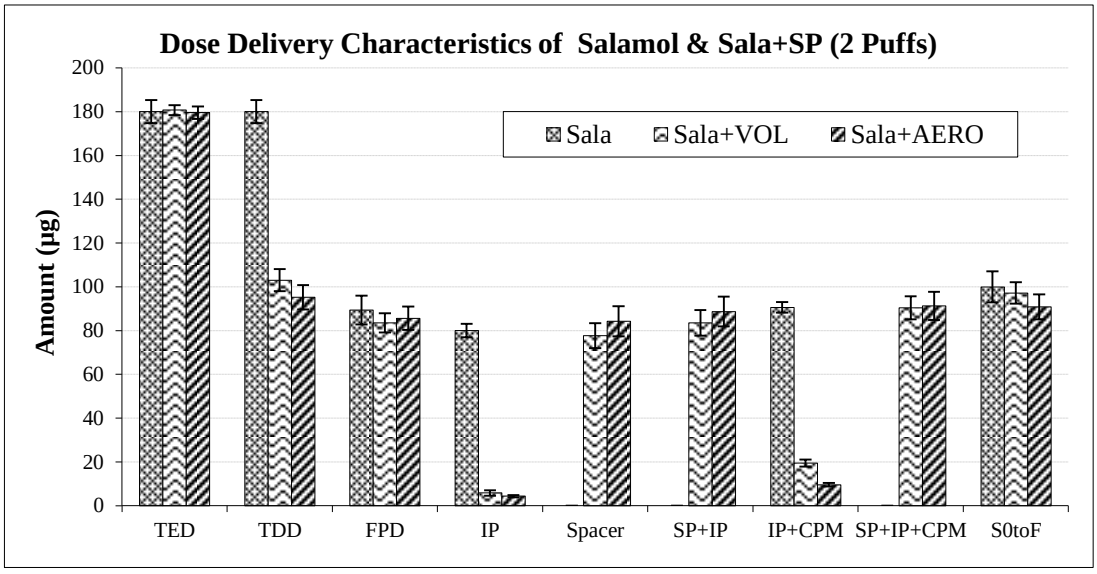


Figure 8.2.3. Dose delivery characteristics of Salamol alone and with spacers.

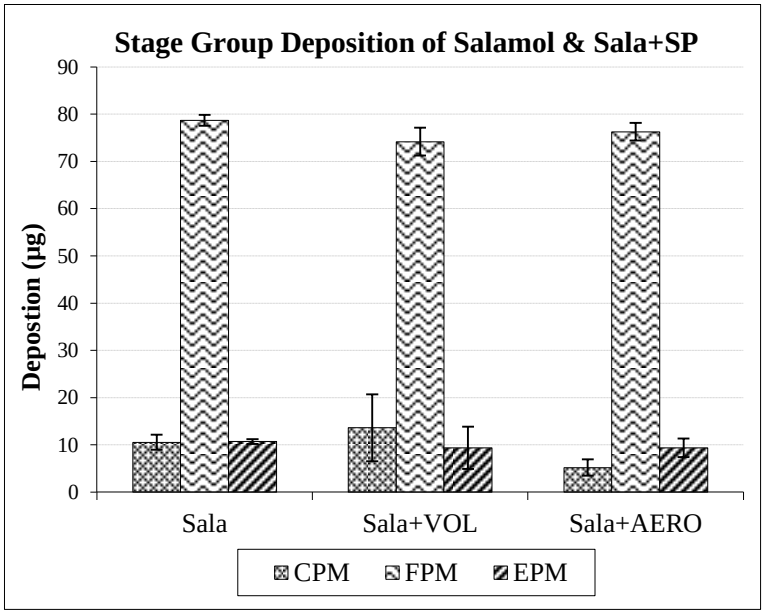


Figure 8.2.4. Stage group deposition of Salamol alone and with spacers.

FPM was statistically similar and *in-vitro* equivalent between the three treatment methods while EPM was only statistically similar (Table 8.2.4 to Table 8.2.6 & Table 8.2.11; Figure 8.2.4). On the other hand, CPM showed significant differences between them and was therefore *in-vitro* equivalent.

The MMAD and GSD were *in-vitro* equivalent between the three treatment methods (Table 8.2.4 & Table 8.2.10). However, statistical similarity was observed only between Salamol Vs Sala+AERO for MMAD and between Salamol Vs Sala+VOL for GSD.

VOL and AERO were 15 and 20 times more efficient than Salamol alone in delivering FPD to the impactor (Table 8.2.8; Figure 8.2.3). However, the ratio of FPD to the combined deposition of emitted dose in the spacer and IP was relatively smaller than the ratio of FPD to IP of the MDI alone. Similar trend was observed when FPD was compared with the non-respirable fraction of the TED, i.e., the combined deposition in IP+CPM and SP+IP+CPM.

Summary of Results

The TED, FPD, FPF (%TED) were statistically similar and *in-vitro* equivalent between the three Salamol treatment methods (MDI alone, Sala+VOL and Sala+AERO). Their MMAD and GSD were also *in-vitro* equivalent. The TDD and FPF (%TDD) were *in-vitro* equivalent between the two spacer treatment methods.

Table 8.2.2. Dose delivery and deposition in ACI of Salamol alone and with spacers.

Treatment Method	TED		TDD		SP		IP		SP+IP		S0toF	
	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD
Salamol	180.3	5.2	180.0	5.2	-	-	80.1	3.1	-	-	99.96	7.1
Sala+VOL	180.7	2.3	103.1	5.1	77.7	5.7	5.9	1.3	83.6	5.8	97.17	4.9
Sala+AERO	179.6	2.8	95.2	5.6	84.3	6.9	4.4	0.5	88.7	6.8	90.84	5.7

Table 8.2.3. Dose delivery and deposition in ACI as %TED and %TDD of Salamol alone and with spacers.

Treatment Method	TDD		SP		IP		SP+IP		S0toF		IP_TDD		S0toF_TDD	
	%TED	SD	%TED	SD	%TED	SD	%TED	SD	%TED	SD	%TDD	SD	%TDD	SD
Salamol	100.00	-	-	-	44.52	2.54	-	-	55.48	2.54	44.52	2.54	55.48	2.54
Sala+VOL	57.02	2.94	42.98	2.94	3.25	0.67	46.22	2.93	53.78	2.93	5.70	1.13	94.30	1.13
Sala+AERO	53.05	3.41	46.95	3.41	2.44	0.28	49.39	3.41	50.61	3.41	4.62	0.63	95.38	0.63

Table 8.2.4. FPD, Stage groups, MMAD and GSD of Salamol alone and with spacers.

Treatment Method	FPD		FPM		EPM		CPM		MMAD		GSD	
	µg	SD	µg	SD	µg	SD	µg	SD	µm	SD		SD
Salamol	89.41	6.58	78.71	7.10	10.70	1.74	10.55	1.60	2.56	0.07	1.62	0.05
Sala+VOL	83.56	4.44	74.18	2.96	9.38	1.87	13.62	1.16	2.76	0.09	1.65	0.05
Sala+AERO	85.65	5.33	76.29	4.47	9.37	1.96	5.19	0.48	2.54	0.14	1.50	0.05

Table 8.2.5. FPD and stage groups as %TED of Salamol alone and with spacers.

Treatment Method	FPF (%)		FPM_ED		EPM_ED		CPM_ED	
	%TED	SD	%TED	SD	%TED	SD	%TED	SD
Salamol	49.62	2.36	43.67	2.84	5.95	1.05	5.86	0.88
Sala+VOL	46.24	2.60	41.05	1.66	5.20	1.07	7.54	0.68
Sala+AERO	47.72	3.19	42.50	2.70	5.22	1.09	2.89	0.29

ED = TED

Table 8.2.6. FPD and stage groups as %TDD of Salamol alone and with spacers.

Treatment Method	FPF (%)		FPM_DD		EPM_DD		CPM_DD	
	%DD	SD	%DD	SD	%DD	SD	%DD	SD
Salamol	49.62	2.36	43.67	2.84	5.95	1.05	5.86	0.88
Sala+VOL	81.08	1.31	72.02	1.42	9.06	1.44	13.22	1.04
Sala+AERO	89.94	0.88	80.13	2.14	9.80	1.68	5.45	0.33

DD = TDD

Table 8.2.7. FPD and stage groups as %S0toF of Salamol alone and with spacers.

Treatment Method	FPD_S0toF[a]		FPM_S0toF[a]		EPM_S0toF[a]		CPM_S0toF[a]	
	% S0toF	SD	% S0toF	SD	% S0toF	SD	% S0toF	SD
Salamol	89.44	1.52	78.70	3.37	10.74	2.02	10.56	1.52
Sala+VOL	85.98	1.07	76.38	1.82	9.60	1.44	14.02	1.07
Sala+AERO	94.29	0.37	84.01	2.02	10.28	1.77	5.71	0.37

[a] S0toF = Impactor mass (deposition on ACI stages S0 to S7 & F)

Table 8.2.8. FPD and S0toF delivery efficiency of Salamol alone and with spacers.

Treatment Method	FPD / IP		FPD / SP+IP		FPD / IP+CPM[a]		FPD / SP+IP+CPM[a]		S0toF / IP		S0toF / SP+IP	
	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD	Ratio	SD
Salamol	1.12	0.12	-	-	0.99	0.09	-	-	1.25	0.13	-	-
Sala+VOL	14.69	2.94	1.01	0.12	4.31	0.38	0.86	0.09	17.08	3.43	1.17	0.14
Sala+AERO	19.79	2.93	0.97	0.13	9.00	0.94	0.92	0.12	20.98	3.03	1.03	0.14

[a] CPM = S0+S1+S2

Table 8.2.9. *In-Vitro* Equivalence and Statistical Significance of CQAs of Salamol alone and with spacers.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p value*	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference[c]	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
TED[a]	Salamol	Sala+VOL	0.99	0.96	1.03	1.000	Yes	-0.35	-3.56	2.87	1.000	Yes
		Sala+AERO	1.00	0.97	1.03	1.000	Yes	0.23	-2.99	3.46	1.000	Yes
	Sala+VOL	Sala+AERO	1.01	0.98	1.04	1.000	Yes	0.59	-2.64	3.81	1.000	Yes
TDD[b]	Salamol	Sala+VOL	1.75	1.63	1.88	<0.0001	No	38.49	33.85	43.13	<0.0001	No
		Sala+AERO	1.89	1.76	2.03	<0.0001	No	42.40	37.76	47.04	<0.0001	No
	Sala+VOL	Sala+AERO	1.08	1.01	1.16	0.062	Yes	3.91	-0.73	8.55	0.112	Yes
FPD	Salamol	Sala+VOL	1.07	0.97	1.18	0.375	Yes	2.93	-1.93	7.78	0.359	Yes
		Sala+AERO	1.04	0.95	1.15	0.947	Yes	1.88	-2.98	6.73	0.910	Yes
	Sala+VOL	Sala+AERO	0.98	0.89	1.08	1.000	Yes	-1.05	-5.90	3.81	1.000	Yes
% FPF (%TED)	Salamol	Sala+VOL	1.07	0.98	1.17	0.227	Yes	3.38	-1.44	8.18	0.225	Yes
		Sala+AERO	1.04	0.95	1.14	0.883	Yes	1.90	-2.91	6.71	0.882	Yes
	Sala+VOL	Sala+AERO	0.97	0.89	1.06	1.000	Yes	-1.48	-6.29	3.33	1.000	Yes
% FPF (%TDD)	Salamol	Sala+VOL	0.61	0.58	0.64	<0.0001	No	-31.47	-34.35	-28.59	<0.0001	No
		Sala+AERO	0.55	0.53	0.58	<0.0001	No	-40.32	-43.20	-37.44	<0.0001	No
	Sala+VOL	Sala+AERO	0.90	0.86	0.94	<0.0001	Yes	-8.85	-11.73	-5.97	<0.0001	No
S0toF	Salamol	Sala+VOL	1.03	0.94	1.13	1.000	Yes	1.39	-3.85	6.64	1.000	Yes
		Sala+AERO	1.10	1.00	1.21	0.095	No	4.56	-0.69	9.80	0.098	Yes
	Sala+VOL	Sala+AERO	1.07	0.97	1.18	0.325	Yes	3.17	-2.08	8.41	0.358	Yes

CQA = Critical Quality Attribute; [a] TED = Ex-Actuator; [b] TDD = Dose delivered into ACI Throat (ex-spacer, for MDI TDD = TED); [c] µg except for %FPF.
CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 8.2.10. *In-Vitro* Equivalence and Statistical Significance of MMAD and GSD of Salamol alone and with spacers.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p value*	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference[a]	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
MMAD	Salamol	Sala+VOL	0.93	0.87	0.98	0.030	Yes	-0.20	-0.38	-0.02	0.027	No
		Sala+AERO	1.01	0.95	1.07	1.000	Yes	0.02	-0.17	0.19	1.000	Yes
	Sala+VOL	Sala+AERO	1.09	1.02	1.15	0.018	Yes	0.22	0.04	0.40	0.017	No
GSD	Salamol	Sala+VOL	0.98	0.94	1.03	1.000	Yes	-0.03	-0.12	0.06	1.000	Yes
		Sala+AERO	1.08	1.03	1.13	0.004	Yes	0.12	0.04	0.21	0.005	No
	Sala+VOL	Sala+AERO	1.10	1.05	1.15	0.001	Yes	0.15	0.07	0.24	0.001	No

[a] µm for MMAD, no units for GSD; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 8.2.11. *In-Vitro* Equivalence and Statistical Significance of Stage group deposition of Salamol alone and with spacers.

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p value*	*In-Vitro Equivalence* (0.85-1.18)	Mean Difference[f]	95% CI		*p* value	Statistical Similarity
				LL	UL				LL	UL		
SP+IP[a]	Salamol	Sala+VOL	0.96	0.87	1.06	0.990	Yes	-1.75	-6.56	3.07	1.000	Yes
		Sala+AERO	0.90	0.82	1.00	0.088	No	-4.33	-9.14	0.49	0.084	Yes
	Sala+VOL	Sala+AERO	0.94	0.85	1.04	0.510	Yes	-2.58	-7.40	2.24	0.488	Yes
IP (Throat)	Salamol	Sala+VOL	13.85	11.31	16.97	<0.0001	No	37.10	35.40	38.80	<0.0001	No
		Sala+AERO	18.36	14.99	22.49	<0.0001	No	37.84	36.15	39.54	<0.0001	No
	Sala+VOL	Sala+AERO	1.33	1.08	1.62	0.018	No	0.75	-0.95	2.44	0.736	Yes
Group 1[b, e] (CPM)	Salamol	Sala+VOL	0.77	0.64	0.92	0.013	No	-1.53	-2.57	-0.50	0.004	No
		Sala+AERO	2.02	1.69	2.42	<0.0001	No	2.68	1.65	3.72	<0.0001	No
	Sala+VOL	Sala+AERO	2.63	2.19	3.14	<0.0001	No	4.21	3.18	5.25	<0.0001	No
Group 2[c, e] (FPM)	Salamol	Sala+VOL	1.06	0.95	1.17	0.637	Yes	2.27	-2.25	6.78	0.564	Yes
		Sala+AERO	1.03	0.93	1.14	1.000	Yes	1.21	-3.30	5.73	1.000	Yes
	Sala+VOL	Sala+AERO	0.97	0.88	1.08	1.000	Yes	-1.05	-5.57	3.46	1.000	Yes
Group 3[d, e] (EPM)	Salamol	Sala+VOL	1.15	0.87	1.51	0.784	No	0.66	-0.98	2.29	0.854	Yes
		Sala+AERO	1.15	0.87	1.51	0.760	No	0.67	-0.97	2.30	0.841	Yes
	Sala+VOL	Sala+AERO	1.00	0.76	1.32	1.000	No	0.01	-1.63	1.64	1.000	Yes

[a] Spacer + Throat (For MDI, SP+IP = IP); [b] Group 1 = S0+S1+S2; [c] Group 2 = S3+S4+S5; [d] Group 3 =S6+S7+Filter; [e] See Table 3.3.2 (Chapter 3); [f] µg.

8.2.7 Discussion: *In-Vitro* Equivalence of Salamol Without and With Spacer

The CQAs of Salamol (Sala) used alone and with Volumatic (VOL) and AeroChamber Plus (AERO) have been discussed separately.

8.2.7.1 TED, TDD and Deposition in IP and SP+IP

The TED of the three treatment methods showed statistical similarity and *in-vitro* equivalence. However, the TDD of Salamol was significantly different than that of the two spacer treatment methods. This is because TDD = TED for the MDI while TDD was 57% and 53% of TED, respectively, for VOL and AERO. Hence, *in-vitro* equivalence was not expected. Both spacers respectively retained 43% and 47% of TED which significantly reduced the proportion of emitted dose entering ACI. This in turn significantly reduced deposition in IP as compared to the MDI. This IP deposition was reduced by 13.6 and 18.3 folds respectively by VOL and AERO, representing 7.3% and 5.5% of IP deposition observed with Salamol MDI alone. This indicates efficient filtration of large particle mass from the emitted dose by the two spacers.

Deposition in IP was statistically similar only between the two spacer treatment methods albeit not being *in-vitro* equivalent (Table 8.2.11). Interestingly, the combined spacer and IP deposition was statistically similar and *in-vitro* equivalent between the three treatment methods, except Salamol Vs Sala+AERO only being *in-vitro* inequivalent. The SP+IP deposition of latter treatment method was slightly higher (~4 µg) even though statistically similar with the MDI and therefore the difference may not be clinically significant. Also, the SP+IP deposition was similar and *in-vitro* equivalent between the two spacer treatment methods. Hence both add-on devices were effective in reducing IP deposition. This is important clinically as it may reduce the incidence of salbutamol related systemic effects in sensitive patients.

8.2.7.2 Spacer Volume and Deposition

VOL and AERO have significantly different internal volumes and dimensions (Table 6.2.13; Section 6.2.7.2). Although about 4% more TED was retained in AERO than VOL, yet TED deposition within them was statistically similar albeit not being *in-vitro* equivalent. Besides, TDD from both of them was *in-vitro* equivalent. This suggests that the spacer with larger volume, dimensions and internal axial distance may not be required for use with Salamol MDI. This assessment is further supported by similar

impactor mass (S0toF) and FPD obtained from both spacers. These outcomes are more likely linked to the formulation of Salamol MDI, which contains ethanol as co-solvent. Since ethanol slows down evaporation (Barry and O'Calaghan, 1997; Ross and Gabrio, 1999; Stein and Myrdal, 2006), it may have reduced the velocity of the emitted plume thereby may also have reduced its cone angle (Hautmann et al., 2013; Johnson et al., 2016; Kunda et al., 2017). The relatively smaller deposition of the emitted dose in VOL than AERO is more likely due to its diamond shape which accounted for the cone angle of the discharged aerosol spray. As a consequence, TDD of VOL was greater than AERO. Nevertheless, similar deposition was found within the two spacers. Hence, the cylindrical dimensions of AERO were equally effective as the diamond shaped VOL in providing adequate space for evaporation of the initial emitted droplets (Figure 2.4.1). Similar findings have been reported for Airomir MDI when attached to VOL and AeroChamber (Mitchell et al., 1999). However, it was also found that FPD, FPF as %TED and FPF as % TDD for Sala+AERO were greater than those obtained from Sala+VOL. These findings indicate that AERO may be a better add-on device for Salamol MDI than VOL. It has been reported that the distance between the MDI mouthpiece placed in the lips and the throat of human adult is up to 10 cm (Brambilla et al., 2011; Hautmann et al., 2013). The internal axial distance of AERO measured in this lab is 9.3 cm. This suggests that an internal axial distance of 9.3 cm should be adequate for the optimal performance of Salamol.

8.2.7.3 Impactor Mass

The impactor mass (S0toF) was in the decreasing rank order of Salamol MDI > Sala+VOL > Sala+AERO. Hence, slightly more dose was delivered to the ACI stages with Salamol alone than with the two spacer treatment methods. However, this difference may not be of clinical significance since their FPD and FPM were statistically similar and *in-vitro* equivalent.

8.2.7.4 Respirable Dose (FPD)

The respirable dose (FPD) was in the decreasing rank order of Sala MDI > Sala+AERO > Sala+VOL. This suggests that where appropriate, Salamol MDI alone could be a better choice for the desired treatment. However, the TDD that entered ACI with the attached spacer constituted over 81% of particles in the FPD range with less than 6% deposition in IP as compared to ~50% and ~45%, respectively, with Salamol alone. This may have significance for efficacy and safety of the chosen treatment method.

The FPD for Salamol is greater than that of Sala+VOL and Sala+AERO. This is in conflict with findings where spacer device attached to an MDI produced higher FPD. Mitchell et al. (1999) reported higher FPD of Airomir with these spacers than that of the MDI. However, von Hollen et al. (2011a & b) and Hatley et al. (2014) reported similar FPD of ProAir HFA without and with AERO. Further, Johnson et al. (2016) reported higher FPD for ProAir HFA than when the MDI was used with LiteAire. These varied and differing results for the FPD obtained without and with the spacer preclude suggestions that the use of a spacer should predispose higher FPD. On the contrary, a higher FPD and a differing APSD profile may raise concerns for efficacy and safety, in particular if these differences are more than 15% (Kelly et al., 2001).

8.2.7.5 FPF %TED and FPF %TDD

The FPF as %TED was in the decreasing rank order of Salamol > Sala+AERO > Sala+VOL. Since TED is similar for these treatment methods, the FPF is reflective of the variability in FPD. However, when FPF was assessed as %TDD, the decreasing ranking order changed to Sala+AERO > Sala+VOL > Salamol. The FPF as %TDD of Salamol is the lowest because for an MDI the TED = TDD which is, respectively, 43% to 47% greater than the spacer treatment methods. This might suggest poor delivery of FPD from Salamol when used without these spacers. Nevertheless, the FPD of the MDI was higher than the two spacer treatment methods. Further, the FPF as %TDD of Sala+AERO was better than Sala+VOL. However, this may not be correct. The data shows that more dose (~4 µg) was delivered from VOL than from AERO while the FPD was only ~1 µg lesser with VOL treatment method. Consequently, this disproportionate relationship resulted in a difference of ~9% in the dose delivery characteristics which is suggestive of Sala+AERO being better of the two spacer treatment methods. This is however misleading given that the two spacer treatment methods were *in-vitro* equivalent even though being statistically different. Therefore, the derived metric of FPF as %TDD would mask the actual performance of a given treatment method (also see Section 6.2.7.5).

Nevertheless, FPF as %TDD may be used as a tool to assess the proportion of the FPD contained in the TDD, proportion of deposition in the spacer and to identify the problem area where improvement would be needed. Therefore, this metric could be used in new product development, problem identification and solving to assist in product improvement.

8.2.7.6 Respirable dose delivery efficiency

The FPD to IP ratio for Sala+VOL and Sala+AERO were respectively ~13 and ~18 times larger than that of the MDI. Also, their ratios of FPD to IP+CPM were ~4 and ~9 times higher than Salamol. This suggests that AERO was relatively more efficient than VOL in removing the coarser portion of the TED. However, the ratio of respirable to non-respirable dose (IP+CPM Vs SP+IP+CPM) suggest that more respirable fraction was delivered with Salamol alone than with VOL or AERO. Similar trend was observed when the ratio of impactor mass (S0toF) to IP and SP+IP were compared with. Although these spacers retained most of the coarse mass and are beneficial in this respect, these findings are suggestive of Salamol being more efficient in FPD delivery as compared to the two spacer treatment methods. The ratio of respirable to non-respirable dose was in the decreasing rank order of Sala > Sala+AERO > Sala+VOL.

8.2.7.7 MMAD and GSD

The MMAD of the three treatment methods was within 2.5 µm to 2.8 µm which shows that salbutamol was delivered in the desired particle size range. The dispersion of these particles was less than 1.7 which indicates consistency of these treatment methods in producing similar MMAD. It is therefore highly likely that the inhaled dose will be delivered to the bronchi and bronchioles to relieve bronchospasm. The *in-vitro* equivalence of FPD is suggestive of their similar efficacy.

The MMAD and GSD were in decreasing rank order of Sala+VOL > Salamol > Sala+AERO. Significantly smaller CPM of Sala+AERO seems to have influenced APSD profile resulting in the lowest MMAD and GSD. The largest MMAD and GSD with Sala+VOL could be due to higher CPM as compared to the other two treatment methods. These findings indicate a possible link between CPM and the two metrics.

8.2.7.8 APSD Stage Groups

The APSD profiles (Figure 8.2.1 & Figure 8.2.4) of the three treatment methods depict that these are centred and nearly running parallel at stages 3-5 while showing similar tailing on stages 6-7 & F. However, the fronting is variable due to variability found on stages 0-2 deposition. These profiles therefore reflect on the statistical and *in-vitro* results with respect to these stage groups.

The FPM similarity and *in-vitro* equivalence suggest that the three treatment methods are more likely to be equally effective in the clinical setting. On the other hand, the significant differences in their CPM may highlight the possibility of varying side effects which may be fewer with AERO. EPM statistical similarity suggests that these treatment methods may equally contribute to systemic effects.

8.2.7.9 Salamol spacer treatment methods

The TED, TDD, FPD, FPF (%TED), FPF (%TDD), S0toF, MMAD and GSD were *in-vitro* equivalent between Sala+VOL and Sala+AERO. The two spacers have shown comparability between them with respect to these CQAs. This study has shown that both of them can be used as an add-on device with Salamol MDI. Whether one can be substituted for the other would be a matter of suitability, choice and convenience. This selection should be supported by pharmacokinetic study and where appropriate with the clinical evidence.

8.2.8 Conclusions: *In-Vitro* Equivalence of Salamol Without and With Spacer

The similarity and in-vitro equivalence of FPD of the three treatment methods (Salamol alone, Sala+VOL and Sala+AERO) is suggestive of their equal clinical effects. The FPD, FPM and S0toF (impactor mass) of Salamol MDI alone were greater than the two spacer treatment methods. This indicates that Salamol MDI alone could be used where appropriate.

Large and small volume spacers were equally effective in delivering *in-vitro* equivalent FPD of Salamol.

The results revealed that TED, FPD, FPF (%TED), MMAD and GSD of Salamol used with VOL and AERO were *in-vitro* equivalent between them. Therefore, these two spacers can be considered as suitable add-on devices for Salamol MDI.

The APSD profiles of the three treatment methods do not meet stage-wise *in-vitro* comparison criteria of EMA (2009) except for FPM.

Findings of this study cannot be extrapolated to other salbutamol MDIs or/and spacers.

8.3 *In-Vivo* Equivalence of Salamol Without and With Spacer-Urinary Pharmacokinetic Studies

The objectives of this study are to investigate *in-vivo* equivalence between Salamol alone and with Volumatic and AeroChamber Plus using urinary pharmacokinetic method developed by Hindle and Chrystyn (1992).

8.3.1 Study Design

Study design is enumerated in Chapter 3 (Sections 3.4.5 & Sub-section 3.4.5.4). Subjects were selected and trained as detailed in Sections 3.4.3 & 3.4.4, respectively. This chapter has adopted similar study design as described in Chapters 5 (Section 5.3.1), 6 (Section 6.3.1) and 7 (Section 7.3.1).

In summary, this study comprised two parts. In Part 1 on a given study day, the subjects inhaled two puffs of primed Salamol, 30 seconds apart, from one of the three treatment methods, vis-à-vis: Salamol alone, Sala+VOL and Sala+AERO. In Part 2, these subjects repeated inhaling salbutamol from one of these treatment methods with the ingestion of charcoal. Each study was separated by 7 days and all subjects inhaled from each of the three Salamol treatment methods on a different study day. All subjects provided blank urine sample 0.5h before inhaling salbutamol dose and then 0.5h post-dose. Thereafter, all urine was pooled for 24 hours.

The pH and volume of samples were recorded which were stored at –20°C before analysis. Post-dose residual salbutamol was recovered from MDI components and spacers.

8.3.2 Sample Analysis

Urine and aqueous samples were processed and assayed for salbutamol using validated HPLC methods described in Chapter 4.

8.3.3 Statistical Analysis

Statistical analysis approach is elaborated in Chapter 3 (Section 3.4.7).

8.3.4 Results: *In-Vivo* Equivalence of Salamol Without and With Spacer

Demographic characteristics of volunteers are given in Table 5.3.1 and Appendix 5.3.4.1 (Chapter 5). Table 8.3.1 to Table 8.3.4 provide summaries of PK metrics

(USAL0.5, USAL24, USAL24Pre, USAL24Post and USALMET) as amounts, % nominal, % delivered and % recovered dose of salbutamol, respectively, for both parts of the study. *In-vivo* equivalence and comparative bioavailability data of these metrics for the three treatment methods are provided in Table 8.3.5 and Table 8.3.6 for Parts 1 and 2 of the study, respectively. These metrics are respectively shown in Figure 8.3.1 to Figure 8.3.5 while their comparative recovery profiles are presented in Figure 8.3.6.

Urinary excretion data of Salamol (MDI alone) for individual subjects is given in Appendices 5.3.4.4 and 5.3.4.7. These data for Sala+VOL and Sala+AERO are shown in Appendices 8.3.4.1 to 8.3.4.4.

Relative lung bioavailability (USAL0.5) of Sala was not *in-vivo* equivalent to any of the two spacer treatment methods (Sala+SP) in both parts of the study (Table 8.3.5 & Table 8.3.6). Although being statistically similar in both parts, these spacer treatment methods were nevertheless only *in-vivo* equivalent to each other when assessed using limits suggested by Parameswaran (1999). USAL0.5 of Sala+SP was about 3 and 2.5 times greater than that of Sala alone in 1st and 2nd Parts of the study, respectively, and hence statistically significant.

Total systemic bioavailability (USAL24NC) in Part 1 of the study was *in-vivo* equivalent as per EMA (2009) criteria and statistically similar between Sala and Sala+SP. However, this was not replicated in Part 2 of the study where USAL24C was *in-vivo* equivalent only between Sala and Sala+AERO as per specifications put forward by Parameswaran (1999). Interestingly, the two spacer treatment methods met EMA (2009) *in-vivo* equivalence criteria between them for total systemic bioavailability in both legs of the study which was also statistically similar.

In Part 1 of the study, the recovery of active (USAL24PreNC) and total salbutamol [(USAL24PostNC) (inclusive of metabolites)] were *in-vivo* equivalent between Sala and Sala+SP to the Parameswaran (1999) criteria. These recoveries were, however, not statistically similar. In Part 2 of the study, the recovery of active salbutamol (USAL24PreC) was neither *in-vivo* equivalent nor statistically similar between Sala and Sala+SP. In contrast, total salbutamol [(USAL24PostC) (inclusive of metabolites)] met *in-vivo* equivalence criteria of Parameswaran (1999) for Sala Vs Sala+SP. The three treatment methods were also statistically similar. Nevertheless, the recovery of salbutamol metabolites (USALMET) in both legs of study was *in-vivo* inequivalent and statistically different between Sala and Sala+SP.

Interestingly, the recoveries of USAL0.5, USAL24, USAL24Pre, USAL24Post and USALMET (except in Part 2) were *in-vivo* equivalent to Parameswaran (1999) criteria and statistically similar between the two spacer treatment methods in both parts of the study (Table 8.3.5 & Table 8.3.6). Further, USAL24NC, USAL24PreNC and USAL24PostNC also conformed to EMA (2009) *in-vivo* equivalence criteria in Part 1 of the study while this was only observed with USAL24C in Part 2 of the study.

Table 8.3.7 and Figure 8.3.7 show the effect of charcoal blockade on each PK parameter of the three treatment methods between Parts 1 and 2 of the study. The results show significant differences between these parameters except for USAL0.5 which was not affected by charcoal intake. The results further reveal that the recovery of salbutamol metabolites (USALMET) was similar between the two parts of the study for the spacer treatment methods even in the presence of charcoal. Table 8.3.8 shows *in-vitro* and *in-vivo* trends of the three Salamol treatment methods.

Table 8.3.1. Mean salbutamol excreted in urine post-inhalation from Salamol without and with spacers.

Treatment Method (n = 13)	**USAL0.5**		**USAL24†**		**USAL24Pre**		**USAL24Post**		**USALMET**	
	µg	SD	µg	SD	µg	SD	µg	SD	µg	SD
Part 1 Study (without charcoal blockade)										
Salamol	6.7	3.2	94.3	19.4	42.9	11.2	87.6	18.4	44.8	12.9
Sala+VOL	19.5	6.2	85.6	10.1	57.5	9.7	66.1	9.7	8.6	5.2
Sala+AERO	17.6	5.9	86.5	13.3	57.4	11.2	68.9	7.7	11.5	5.1
Part 2 Study (with charcoal blockade)										
Salamol	7.2	3.4	50.2	13.1	27.2	11.6	43.0	13.5	15.7	8.9
Sala+VOL	17.5	3.0	66.1	9.6	40.0	7.0	48.6	7.0	8.6	2.3
Sala+AERO	16.6	4.7	62.1	10.2	36.8	7.1	45.5	5.9	8.7	3.6

† TRD = USAL24; SD = Standard Deviation

Table 8.3.2. Mean salbutamol excretion in urine post-inhalation from Salamol without and with spacers, expressed as % of Nominal Dose.

Treatment Method (n = 13)	USAL0.5		USAL24†		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Salamol	3.4	1.6	47.2	9.7	21.4	5.6	43.8	9.2	22.4	6.5
Sala+VOL	9.7	3.1	42.8	5.0	28.8	4.8	33.1	4.8	4.3	2.6
Sala+AERO	8.8	3.0	43.2	6.7	28.7	5.6	34.5	3.8	5.8	2.5
Part 2 Study (with charcoal blockade)										
Salamol	3.6	1.7	25.1	6.5	13.6	5.8	21.5	6.8	7.9	4.5
Sala+VOL	8.8	1.5	33.1	4.8	20.0	3.5	24.3	3.5	4.3	1.1
Sala+AERO	8.3	2.3	31.0	5.1	18.4	3.5	22.8	2.9	4.3	1.8

† TRD = USAL24; SD = Standard Deviation

Table 8.3.3. Mean salbutamol excretion urine post-inhalation from Salamol without and with spacers, expressed as % of estimated Delivered Dose.

Treatment Method (n = 13)	USAL0.5		USAL24†		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)										
Salamol	3.9	1.9	54.2	11.4	24.6	6.4	50.3	10.8	25.7	7.5
Sala+VOL	18.1	5.7	79.3	6.9	53.3	8.3	61.2	7.3	7.9	4.8
Sala+AERO	17.4	4.0	86.9	4.1	57.5	6.1	69.5	2.0	12.0	6.2
Part 2 Study (with charcoal blockade)										
Salamol	4.2	2.0	29.0	7.8	15.7	6.6	24.8	8.0	9.1	5.3
Sala+VOL	16.6	2.5	62.8	7.9	37.9	5.9	46.1	5.9	8.2	2.2
Sala+AERO	17.5	4.2	66.0	8.1	39.1	5.8	48.5	4.4	9.4	4.0

† TRD = USAL24; SD = Standard Deviation

Table 8.3.4. Mean salbutamol excretion in urine post-inhalation from Salamol without and with spacers, expressed as % of Recovered Dose.

Treatment Method (n = 13)	USAL0.5		USAL24Pre		USAL24Post		USALMET	
	%	SD	%	SD	%	SD	%	SD
Part 1 Study (without charcoal blockade)								
Salamol	7.1	3.1	45.4	9.4	92.9	3.1	47.5	8.7
Sala+VOL	22.8	6.8	67.1	7.2	77.2	6.8	10.2	6.5
Sala+AERO	19.8	3.8	66.1	5.4	80.2	3.8	14.1	7.7
Part 2 Study (with charcoal blockade)								
Salamol	15.8	11.3	53.2	16.0	84.2	11.3	31.0	14.3
Sala+VOL	26.5	2.1	60.3	3.3	73.5	2.1	13.2	3.6
Sala+AERO	26.3	3.3	59.3	4.9	73.7	3.3	14.5	6.7

TRD = USAL24; SD = Standard Deviation

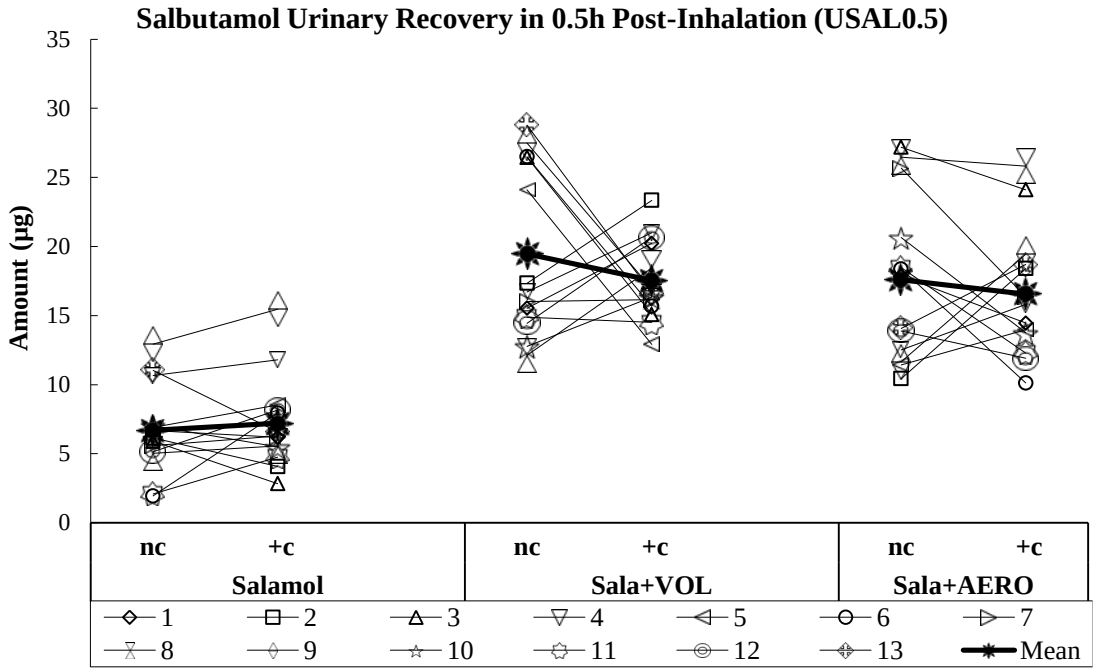


Figure 8.3.1. Comparative salbutamol urinary excretion at 0.5h post-inhalation of Salamol without and with spacers.

Numerals represent individual volunteers. nc = no charcoal ingestion; +c = with charcoal ingestion.

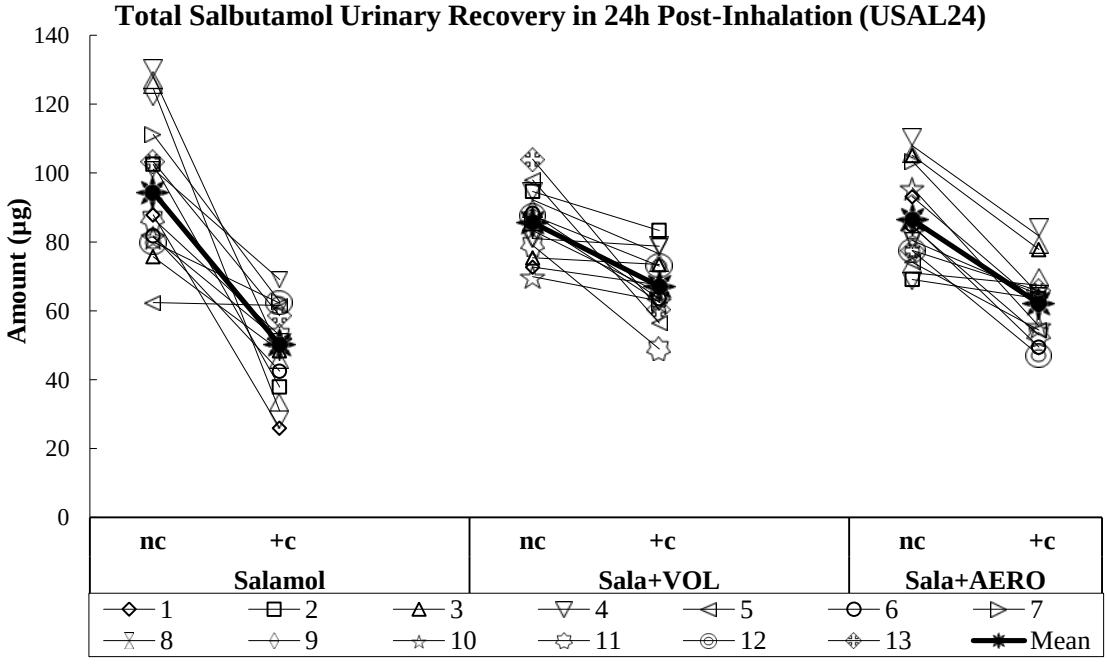


Figure 8.3.2. Comparative total salbutamol urinary excretion during 24h post-inhalation of Salamol without and with spacers.

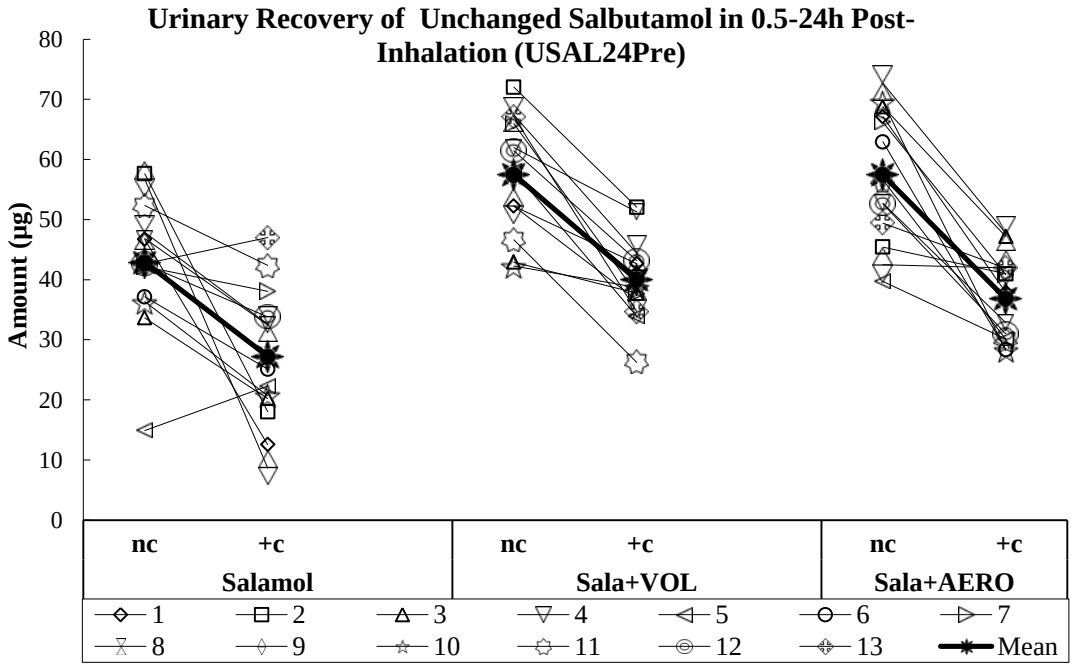


Figure 8.3.3. Comparative unchanged salbutamol urinary excretion during 0.5-24h post-inhalation of Salamol without and with spacers.

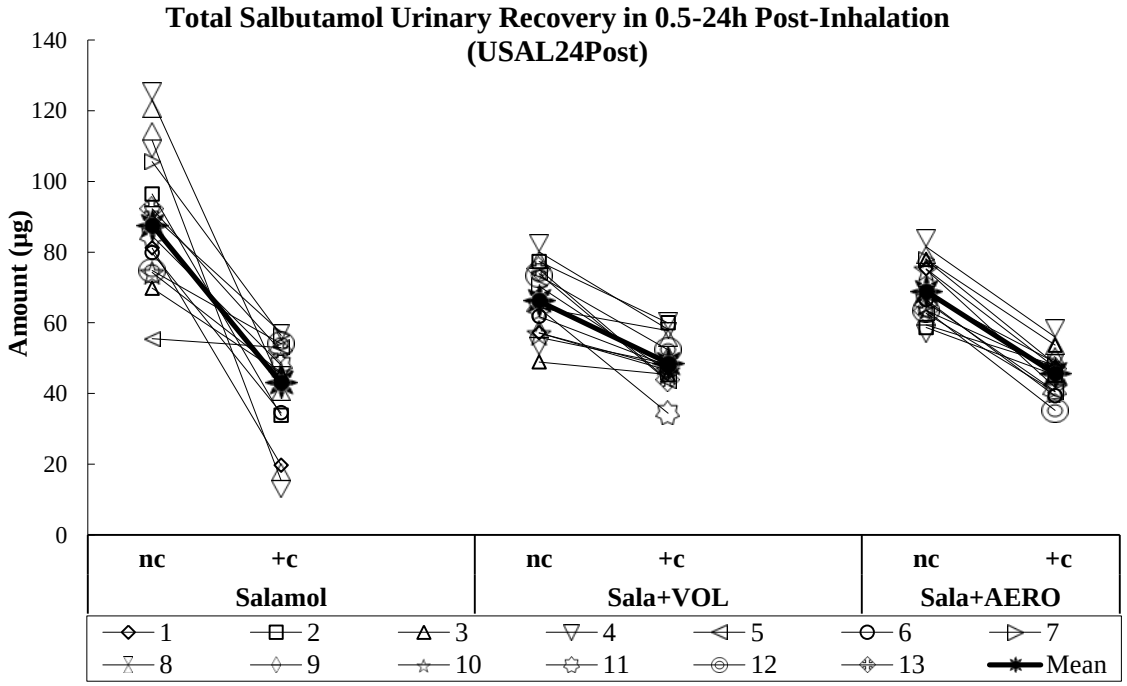


Figure 8.3.4. Comparative total salbutamol urinary excretion during 0.5-24h post-inhalation of Salamol without and with spacers.

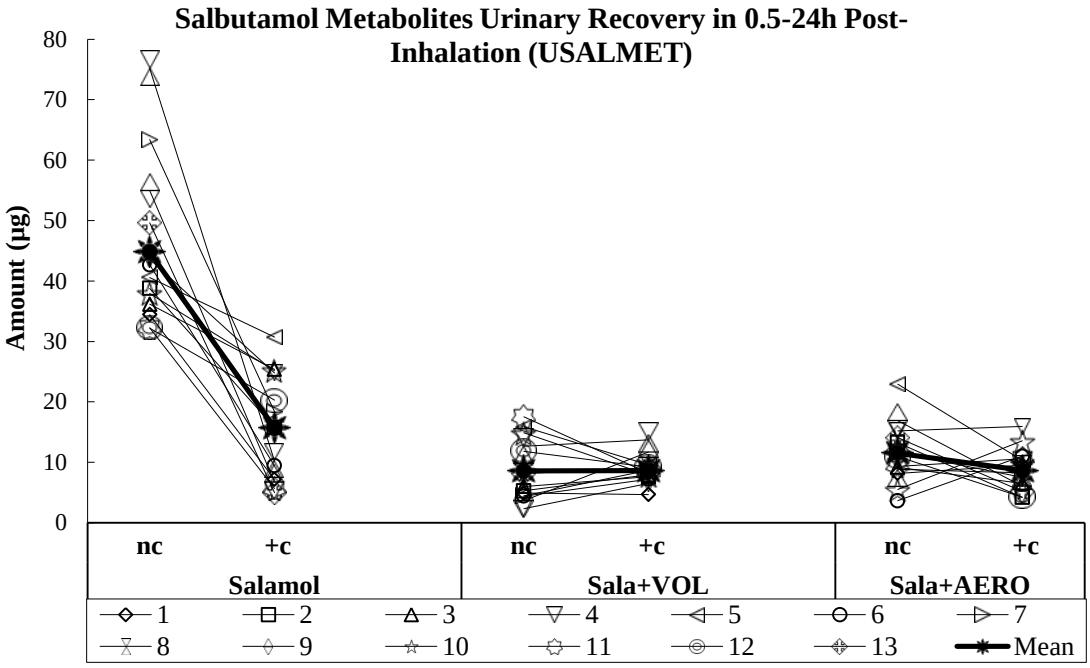


Figure 8.3.5. Comparative salbutamol metabolites urinary excretion during 0.5-24h post-inhalation of Salamol without and with spacers.

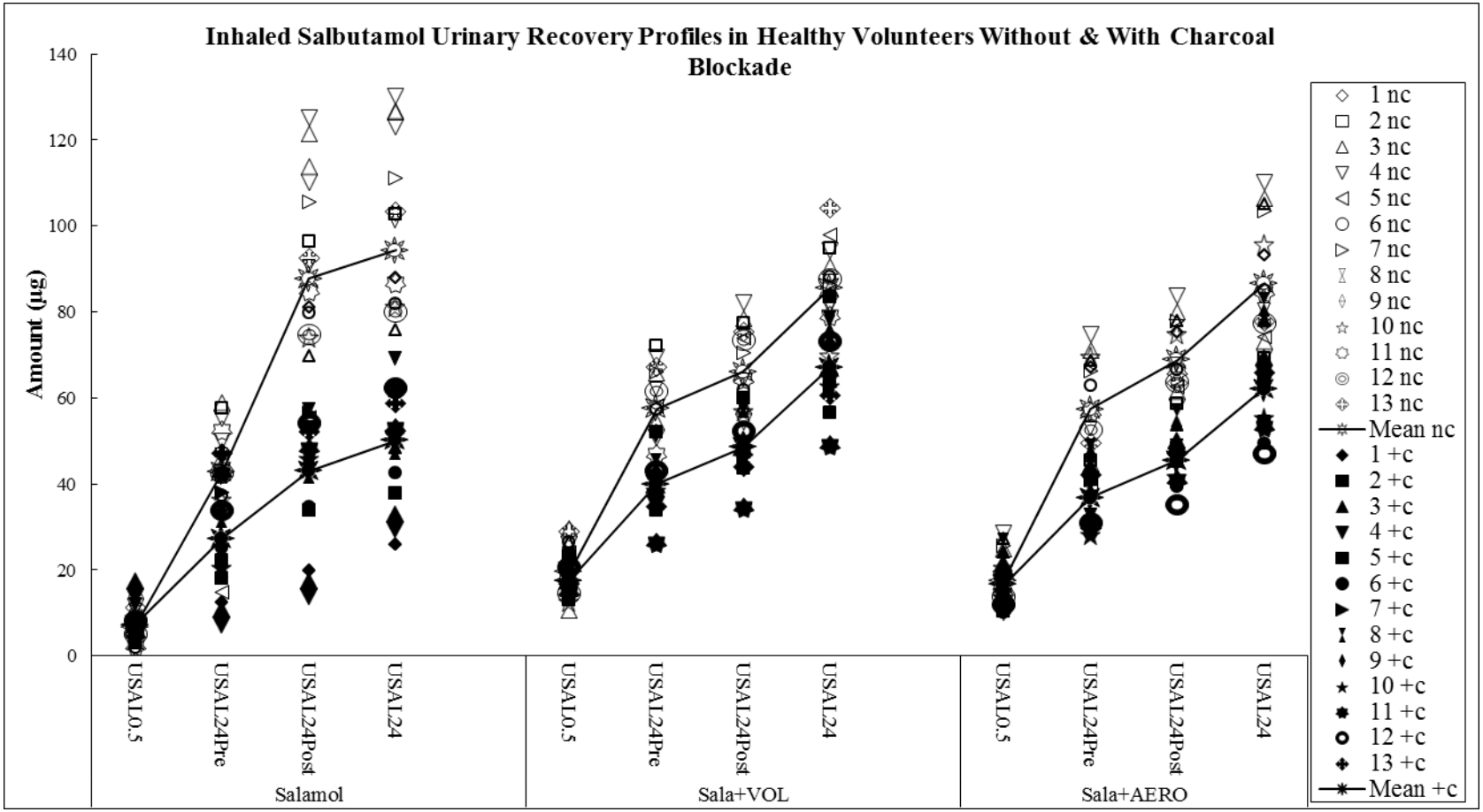


Figure 8.3.6. Comparative salbutamol urinary recovery profiles obtained post-inhalation of Salamol without and with spacers.

Numerals represent individual volunteers. nc = no charcoal ingestion; +c = with charcoal ingestion.

Table 8.3.5. *In-Vivo* Equivalence and Statistical Significance of Salamol without and with spacers (Part 1 Study).

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p* value	*In-Vivo* Equivalence		Mean Difference[c]	95% CI		*p* value	Statistical Similarity
				LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
USAL0.5NC	Salamol	Sala+VOL	0.32	0.24	0.43	<0.0001	No	No	-12.77	-17.30	-8.24	<0.0001	No
		Sala+AERO	0.35	0.26	0.48	<0.0001	No	No	-10.89	-15.42	-6.36	<0.0001	No
	Sala+VOL	Sala+AERO	1.11	0.83	1.50	0.541	No	Yes	1.89	-2.64	6.42	0.398	Yes
USAL24NC	Salamol	Sala+VOL	1.09	0.97	1.21	0.205	Yes	Yes	8.75	-3.16	20.66	0.142	Yes
		Sala+AERO	1.08	0.97	1.21	0.239	Yes	Yes	7.85	-4.06	19.76	0.186	Yes
	Sala+VOL	Sala+AERO	0.99	0.89	1.11	0.925	Yes	Yes	-0.90	-12.81	11.01	0.877	Yes
USAL24PreNC	Salamol	Sala+VOL	0.80	0.69	0.91	0.008	No	Yes	-11.55	-20.19	-2.91	0.011	No
		Sala+AERO	0.80	0.70	0.92	0.010	No	Yes	-11.44	-20.08	-2.79	0.012	No
	Sala+VOL	Sala+AERO	1.01	0.88	1.15	0.933	Yes	Yes	0.12	-8.53	8.76	0.978	Yes
USAL24PostNC	Salamol	Sala+VOL	1.31	1.18	1.46	0.000	No	Yes	21.53	11.83	31.23	<0.0001	No
		Sala+AERO	1.25	1.13	1.39	0.001	No	Yes	18.74	9.04	28.44	0.001	No
	Sala+VOL	Sala+AERO	0.96	0.86	1.06	0.461	Yes	Yes	-2.79	-12.49	6.91	0.558	Yes
USALMETnc	Salamol	Sala+VOL	5.52	3.82	7.99	<0.0001	No	No	33.08	24.80	41.36	<0.0001	No
		Sala+AERO	3.79	2.62	5.48	<0.0001	No	No	30.18	21.90	38.45	<0.0001	No
	Sala+VOL	Sala+AERO	0.69	0.47	0.99	0.093	No	No	-2.91	-11.18	5.37	0.476	Yes

[a] EMA, 2009; [b] Parameswaran, 1999; [c] µg; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 8.3.6. *In-Vivo* Equivalence and Statistical Significance of Salamol without and with spacers (Part 2 Study).

Parameter	Multiple Comparisons		Mean Ratio	90% CI		*p* value	*In-Vivo* Equivalence		Mean Difference[c]	95% CI		*p* value	Statistical Similarity
				LL	UL		0.80-1.25[a]	0.67-1.50[b]		LL	UL		
USAL0.5C	Salamol	Sala+VOL	0.38	0.31	0.47	<0.0001	No	No	-10.33	-13.39	-7.28	<0.0001	No
		Sala+AERO	0.41	0.33	0.51	<0.0001	No	No	-9.35	-12.40	-6.30	<0.0001	No
	Sala+VOL	Sala+AERO	1.08	0.87	1.35	0.541	No	Yes	0.98	-2.07	4.03	0.514	Yes
USAL24C	Salamol	Sala+VOL	0.73	0.63	0.84	0.001	No	No	-16.96	-25.84	-8.08	0.001	No
		Sala+AERO	0.79	0.68	0.91	0.009	No	Yes	-11.91	-20.79	-3.03	0.011	No
	Sala+VOL	Sala+AERO	1.08	0.94	1.25	0.346	Yes	Yes	5.05	-3.83	13.93	0.252	Yes
USAL24PreC	Salamol	Sala+VOL	0.63	0.50	0.78	0.002	No	No	-12.76	-20.18	-5.33	0.002	No
		Sala+AERO	0.68	0.54	0.85	0.007	No	No	-9.62	-17.05	-2.19	0.013	No
	Sala+VOL	Sala+AERO	1.09	0.87	1.36	0.527	No	Yes	3.14	-4.29	10.56	0.392	Yes
USAL24PostC	Salamol	Sala+VOL	0.84	0.70	1.00	0.104	No	Yes	-5.63	-13.35	2.10	0.146	Yes
		Sala+AERO	0.89	0.74	1.07	0.287	No	Yes	-2.56	-10.29	5.17	0.501	Yes
	Sala+VOL	Sala+AERO	1.07	0.89	1.28	0.555	No	Yes	3.07	-4.66	10.80	0.421	Yes
USALMETc	Salamol	Sala+VOL	1.58	1.16	2.17	0.020	No	No	7.13	2.61	11.65	0.003	No
		Sala+AERO	1.65	1.21	2.26	0.012	No	No	7.06	2.54	11.59	0.004	No
	Sala+VOL	Sala+AERO	1.04	0.76	1.43	0.822	No	Yes	-0.07	-4.59	4.45	0.975	Yes

[a] EMA, 2009; [b] Parameswaran, 1999; [c] µg; CI = Confidence Interval; LL = Lower limit; UL = Upper Limit

Table 8.3.7. Statistical comparison of salbutamol post-inhalation urinary excretion between Part 1 and Part 2 studies of Salamol without and with spacers.

Parameter[1] [nc Vs (+c)]	**Treatment**	**Mean paired Difference**[2]	**95% CI**		***t value***	***p value***	**Statistical Similarity**
			LL	**UL**			
USAL0.5	Salamol	-0.50	-2.22	1.21	-0.640	0.534	Yes
	Sala+VOL	1.94	-2.84	6.72	0.884	0.394	Yes
	Sala+AERO	1.04	-2.51	4.59	0.636	0.537	Yes
USAL24	Salamol	44.18	28.76	59.60	6.243	<0.0001	No
	Sala+VOL	18.47	10.12	26.82	4.819	<0.0001	No
	Sala+AERO	24.42	17.10	31.75	7.264	<0.0001	No
USAL24Pre	Salamol	15.66	5.91	25.42	3.500	0.004	No
	Sala+VOL	17.54	12.34	22.73	7.358	<0.0001	No
	Sala+AERO	20.56	13.28	27.83	6.158	<0.0001	No
USAL24Post	Salamol	44.68	29.27	60.09	6.318	<0.0001	No
	Sala+VOL	17.53	11.69	23.37	6.542	<0.0001	No
	Sala+AERO	23.39	19.43	27.34	12.893	<0.0001	No
USALMET	Salamol	29.02	18.57	39.47	6.052	<0.0001	No
	Sala+VOL	-0.01	-3.04	3.02	-0.008	0.994	Yes
	Sala+AERO	2.83	-1.18	6.84	1.538	0.150	Yes

[1] nc = no charcoal; +c = with charcoal; [2] µg

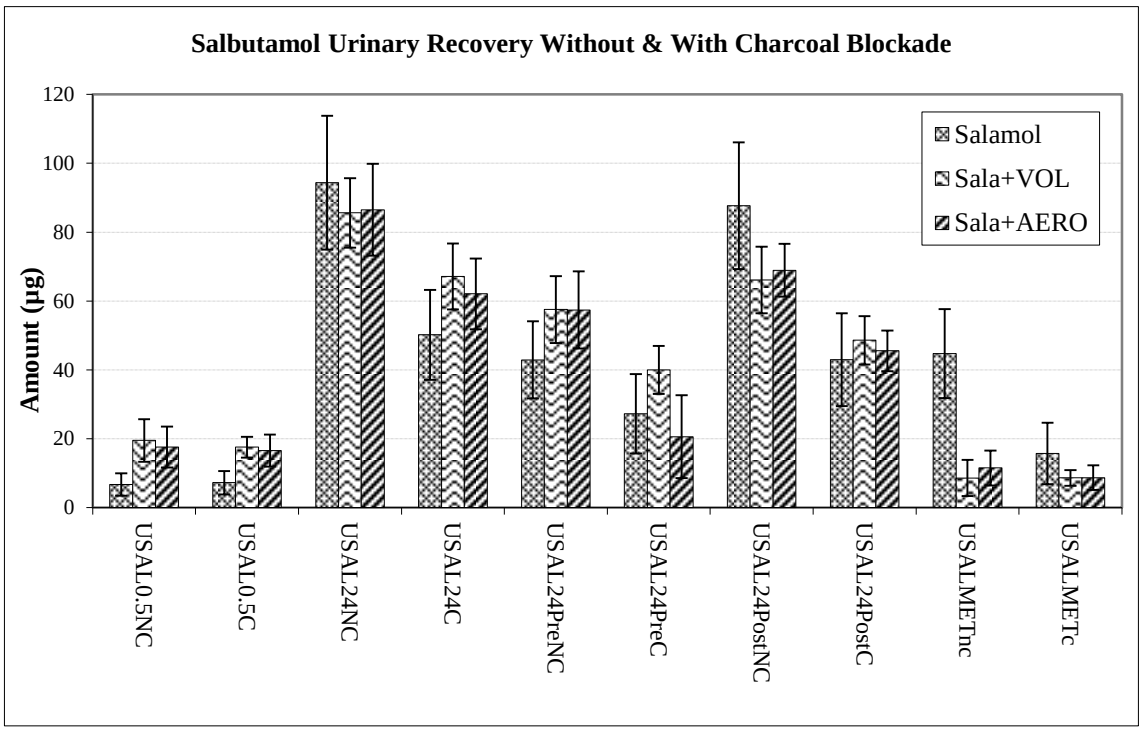


Figure 8.3.7. Comparison of salbutamol post-inhalation urinary excretion of Salamol without and with spacers between Part 1 and Part 2 (with charcoal blockade) studies.

Table 8.3.8. *In-vitro* and *in-vivo* trends between APSD performance metrics and salbutamol post-inhalation urinary excretion of Salamol without and with spacers.

Parameter	Salamol	Sala+VOL	Sala+AERO	Trend (in decreasing order)
	µg	µg	µg	
FPD	89.4	83.6	85.7	Sala > Sala+AERO > Sala+VOL
%FPF (%TDD)	49.6%	81.1%	89.9%	Sala+AERO > Sala+VOL > Sala
S0toF	100.0	97.2	90.8	Sala > Sala+VOL > Sala+AERO
USAL0.5NC	6.7	19.5	17.6	Sala+VOL > Sala+AERO > Sala
USAL0.5C	7.2	17.5	16.6	Sala+VOL > Sala+AERO > Sala
IP+CPM	90.6	19.5	9.6	Sala > Sala+VOL > Sala+AERO
IP	80.1	5.9	4.4	Sala > Sala+VOL > Sala+AERO
USAL24PreNC	42.9	57.5	57.4	Sala+VOL > Sala+AERO > Sala
USAL24PreC	27.2	40.0	36.8	Sala+VOL > Sala+AERO > Sala
USAL24PostNC	87.6	66.1	68.9	Sala > Sala+AERO > Sala+VOL
USAL24PostC	43.0	48.6	45.5	Sala+VOL > Sala+AERO > Sala
TDD (ACI)	180.0	103.2	95.2	Sala > Sala+VOL > Sala+AERO
TDD (NC)	174.2	107.9	99.2	Sala > Sala+VOL > Sala+AERO
TDD (C)	173.6	105.2	93.7	Sala > Sala+VOL > Sala+AERO
USAL24NC	94.3	85.6	86.5	Sala > Sala+AERO > Sala+VOL
USAL24C	50.2	66.1	62.1	Sala > Sala+VOL > Sala+AERO

8.3.5 Discussion: *In-Vivo* Equivalence of Salamol Without and With Spacer

Volumatic (VOL) is the recommended spacer for Salamol (Teva, 2016) while AeroChamber Plus (AERO) is promoted for use with it. The urinary PK data indicate that the two spacers significantly increased the relative lung bioavailability of salbutamol (USAL0.5) from Salamol as compared to the MDI alone. This rendered the two treatment methods (Sala Vs Sala+SP) *in-vivo* inequivalent. However, the increase in lung bioavailability may be of concern for safety in hypersensitive patients. Nevertheless, Lipworth et al. (1988) have shown in asthmatics that the side effects of salbutamol MDI were well tolerated and infrequent with cumulative doses from 100 to 4000 µg. Hence, spacer mediated increase in salbutamol bioavailability may be beneficial in relieving the acute asthma attack, in particular in patients who have difficulty in inhaling salbutamol from the MDI alone.

Intriguingly, the *in-vivo* relative lung deposition (USAL0.5) in healthy volunteers is in conflict with that of *in-vitro* FPD (respirable dose) obtained with ACI for the MDI and the two spacer treatment methods (Table 8.2.4 & Table 8.2.9). FPD is considered as the proportion of the inhaled puff that is highly likely to deposit in the lungs (Newman, 1998; Chrystyn et al., 2015) and was *in-vitro* equivalent and statistically similar for the

three Salamol treatment methods. The conflicting results are more likely due to differences in the two systems (*in-vitro* Vs *in-vivo*), having different aerodynamics and environments.

In ACI, the airflow is constant and maintained at 28.3 L/min under controlled conditions. In human respiratory tract (HRT), however, the inhalation manoeuvre is affected by aerosol impaction in the oropharyngeal cavity such as reflexive cessation of inhalation (Crompton, 1982). Besides, the anatomy and physiology play a significant role in this mechanism (Labiris and Dolovich, 2003). It is also likely that spacers may have comforted volunteers with their inhalation technique besides eliminating the reflex cessation of inhalation. The increased relative lung deposition with spacers, therefore, highlights the importance of their use.

The increase in relative lung deposition (USAL0.5NC) was of greater magnitude with Volumatic (2.9 fold) than with AeroChamber Plus (2.6 fold). However, USAL0.5NC was statistically similar between the two spacers. Further, USAL0.5 with VOL differed by <1 µg than that of AERO in both parts of the study and, therefore, may not be clinically significant. Hence, it can be concluded that spacer dimension or volume did not affect lung deposition of Salamol. This is consistent with the *in-vitro* results (Sections 8.2.7.2 & 8.2.7.9). These results are also in line with those observed with Ventolin Evohaler for the two spacers (Table 6.3.1, Table 6.3.9 & Table 6.3.10).

On another note, it is clear that the treatment method using Salamol alone is not *in-vivo* equivalent to the two treatment methods using VOL and AERO. This may have clinical implications. Further, there was little difference between the two Salamol spacer treatment methods.

The relative lung deposition (USAL0.5C) patterns were reproducible in Part 2 Study (charcoal blockade) for three Salamol treatment methods (Table 8.3.1 & Table 8.3.6). Further, the comparative relative lung deposition of these treatment methods was statistically similar between the two parts of the study (Table 8.3.7, Figure 8.3.7). Interestingly, these results also reflect on the trend found with Ventolin Evohaler with the two spacers (Table 6.3.1 & Table 6.3.10). These findings clearly re-inforce that the excretion of inhaled salbutamol in the first 0.5h mainly comes from the dose deposited in the lungs.

The total systemic bioavailability (USAL24NC) was *in-vivo* equivalent and statistically similar between the three treatment methods in Part 1 of the study (Table 8.3.1 & Table 8.3.5). This suggests that USAL24NC was indifferent to the method of salbutamol delivery from Salamol. However, in Part 2 study (charcoal blockade), greater total systemic recoveries (USAL24C) from Sala+VOL and Sala+AERO indicate that spacer method of delivery was more efficient than the MDI alone (Table 8.3.2 & Table 8.3.3). This also pinpoints that the systemic delivery (USAL24C) in the presence of charcoal blockade of GIT absorption was mainly from the lung deposition and that the greater lung deposition with the two spacers (USAL0.5C) was responsible for greater total systemic bioavailability (Table 8.3.1). This is further evident from the higher contribution of lung deposition when assessed as % estimated delivered dose (Table 8.3.3) and its proportion in the total systemic bioavailability (Table 8.3.4).

The total systemic bioavailability with charcoal blockade (USAL24C) was about 53%, 77% and 72% for Salamol, Sal+VOL and Sala+AERO, respectively, to that without charcoal ingestion (Table 8.3.1). This indicates that about half of the inhaled dose was swallowed with the MDI while this swallowed proportion was less than 28% when spacers were used. The two spacers therefore effectively reduced oropharyngeal deposition. The total systemic bioavailability with charcoal blockade has been mainly derived from the lung deposition (USAL0.5C). USAL24C represented 29%, 63% and 66%, respectively, of the estimated delivered dose (TDD) which was predominantly derived from USAL0.5C constituting 4%, 17% and 18% of TDD.

The *in-vitro* TDD and *in-vivo* estimated TDD (ND-UDD) reflect on differences of USAL24 between the two parts of the study (Table 8.3.8). However, USAL24NC is similar to *in-vitro* S0toF (impactor mass) which is the dose that reached to the ACI, a surrogate for the human respiratory system. This indicates that oropharyngeal deposition may have removed a large proportion of the dispensed dose when Salamol alone was used. This is evident from the 45% *in-vitro* recovery of the TED from IP (Table 8.2.3). On the other hand, similar %TED is retained by the two spacers while significantly reducing IP deposition. This may have allowed more of the dispensed dose in the fine particle range (FPD) to reach lungs. Indeed, *in-vitro* studies indicated that Salamol had 50% FPF as compared to 81% and 90% for Sala+VOL and Sala+AERO, respectively (Table 8.2.6). Further, *in-vitro* S0toF made 56%, 94% and 95%, respectively, of the TDD for these treatment methods (Table 8.2.3) and reflects on

greater systemic bioavailability (USAL24C) observed in charcoal blockade study. These findings highlight the predictive value of *in-vitro* studies for *in-vivo* metrics.

In Part 1 Study, *in-vivo* equivalence to Parameswaran's (1999) criteria was observed for unchanged active salbutamol (USAL24PreNC) between Salamol and the two spacer treatment methods (Sala+SP). However, this was not observed with USAL24PreC in Part 2 Study. Further, USAL24Pre was statistically different in both parts of the study between them. Unchanged salbutamol is mainly derived from lung deposition (Shenfield et al., 1976; Tomlinson et al., 1995; Ward et al., 2000). Greater lung deposition (USAL0.5) reflected on higher amounts of unchanged salbutamol excreted in urine over 24 hours in both parts of the study (Table 8.3.1 to Table 8.3.4). USAL24PreC formed 63%, 70% and 64% of USAL24PreNC for Salamol, Sala+VOL and Sala+AERO, respectively. This indicates that these proportions of the unchanged salbutamol were mainly derived from the lung deposition since absorption from GIT was blocked with the ingestion of activated charcoal. Hence, in Part 1 Study, the remaining proportion of salbutamol may have escaped biotransformation, entered systemic circulation and was excreted in urine as unchanged salbutamol (

Figure 2.3.4, Chapter 2) (Chrystyn, 2000; Ward et al., 2000).

The results suggest that urinary excretion of unchanged salbutamol over 24 hour (USAL24Pre) can be anticipated from lung deposition (USAL0.5). In Part 1 Study, USAL0.5NC formed 16%, 34% and 31% of USAL24PreNC, respectively, of the three treatment methods. In Part 2 Study, 27%, 44% and 45% of USAL24PreC may have originated from USAL0.5C. The urinary excretion of unchanged salbutamol from Salamol used with the two spacers is statistically similar within each part of the study (Table 8.3.5 & Table 8.3.6). Interestingly, this phenomenon is consistent across the two parts of the study for each of the three treatment methods when their respective relative proportions of USAL24PreNC (63%, 70% and 64%) are taken into account.

The presence of unchanged pharmacologically active salbutamol (USAL24Pre) would reflect on the effectiveness of the responsive bronchodilatory treatment for the duration of dosing interval. Larger bioavailability of USAL24Pre may also predispose salbutamol related systemic effects in some hypersensitive patients. Further, the results suggest that Salamol when used with VOL and AERO may provide better and consistent effects than the MDI alone.

The urinary excretion of metabolised salbutamol from Salamol in Part 1 Study was 2.9 folds more than that found in Part 2 Study. Salbutamol is primarily metabolised in the liver (Ward et al., 2000) and a large proportion of the orally absorbed drug undergoes first pass degradation. The co-administration of charcoal prevents GIT absorption, hence, a larger and significant difference in the recovery of salbutamol metabolites was observed between the two parts of the study. The recovery of metabolites with charcoal ingestion also indicates that about 35% of salbutamol absorbed from the lungs was metabolised over 24 hours. This may have occurred mainly in the liver since first-pass conjugation has not been reported in the lungs (Shenfield et al., 1976; Olsson et al., 2011). About 8% of salbutamol is also protein bound (Martin et al., 1971; Goldstein et al., 1987) which may also have been metabolised and contributed to the total recovery of metabolites.

On the other hand, USALMET was statistically similar for Sala+SP between the two parts of the study. This indicates that with the spacer treatment method, the metabolites excreted in urine from systemic circulation originated mainly from the lungs deposition. This is evident from their *in-vivo* equivalence to Parameswaran's (1999) criteria in the charcoal blockade which was not observed in the 1st part of the study and is more likely due to individual variation in GIT absorption of the swallowed salbutamol.

The two spacers significantly reduced the systemic availability of Salamol dose; VOL and AERO respectively retained 40% and 45% of the TED in Part 1 Study. These values were 42% and 48% in Part 2 Study. This dose retention in spacers is reflective of their volumes and is similar to those reported for *in-vitro* studies (Table 8.2.2 & Table 8.2.3). Interestingly, similar fraction of dose was retained when VOL was attached to Ventolin Evohaler which was at 42% and 41% of TED in Parts 1 and 2 of the study, respectively. However, more TED of Ventolin Evohaler was retained in AERO and these values were 50% and 51%. This is more likely due to the fast spray speed and greater ballistic force of Evo resulting in higher deposition in AERO (Brambilla et al., 2011; Hautmann et al., 2013; Johnson et al., 2016; Kunda et al., 2017). On the other hand, 44% of Airomir TED was retained in AERO in both parts of the study which is lower than that of Sala+AERO. These differences reflect on the differences in formulation and device design of the three MDIs which produce emitted dose with differing ballistic characteristics of their sprays (Brambilla et al., 2011; Hautmann et al., 2013; Johnson et al., 2016; Kunda et al., 2017).

The co-administration of charcoal significantly blocked the absorption of the swallowed portion of salbutamol dose of the three Salamol treatment methods (Table 8.3.7). However, urinary excretion of salbutamol in 0.5 hour (USAL0.5), a surrogate for the delivery to the lungs, was statistically similar in both parts of the study. This re-affirms that USAL0.5 is an index of lung deposition which is unaffected by charcoal ingestion. These results also confirm the findings with Ventolin Evohaler (Table 6.3.9) and Airomir (Table 7.3.6). Further, these observations also re-affirm that charcoal co-administration effectively differentiates between the lung and systemic absorption of inhaled drugs (Borgström and Nilsson, 1990).

8.3.5.1 *In-Vitro* and *In-Vivo* trends

FPD of Salamol did not reflect on the USAL0.5 (Table 8.3.8). FPD was *in-vitro* equivalent and statistically similar between the MDI and the two spacer treatment methods while this was not observed with *in-vivo* studies for USAL0.5. Although lung deposition (USAL0.5) with the two spacer treatment methods was *in-vivo* equivalent and statistically similar between them, yet this was significantly greater than the MDI in both parts of the study. Nevertheless, FPD as a fraction of delivered dose (%FPF) broadly represented the differences of USAL0.5 between the MDI and spacer treatment methods. As pointed out earlier, the significant increase in USAL0.5 with the spacer treatment method may have been partly due to the good control of inhalation manoeuvre. Moreover, the absence of throat impaction effect, causing reflexive cessation of inhalation manoeuvre, may have contributed to this difference, which is evident from the significantly reduced IP deposition with the two spacers. The significant decrease in IP deposition and non-respirable dose (IP+CPM) with spacer treatment methods as compared to the MDI is reflected in significant increase in active salbutamol (USAL24Pre) and decrease in metabolites (USALMET) over 24 hours.

The spacer removes a significant amount of the TED; hence, TDD is expected to be significantly different than that of the MDI alone. This was observed *in-vitro*. However, their total systemic bioavailability (USAL24NC) in Part 1 Study was *in-vivo* equivalent and statistically similar. Given that a spacer removes large proportion of the TED, it is rationale to compare the impactor mass (S0toF) to USAL24NC. This comparison reveals similarity of *in-vitro* trend to that of *in-vivo*. Nonetheless, due to the blockade of salbutamol absorption in Part 2 Study, significant increase was observed in USAL24C

with the two spacers as compared to the MDI alone. Although being in contrast to TDD, this mimics the trend observed with the lung deposition (USAL0.5C) which is the main source of systemic bioavailability when GIT absorption is prevented with charcoal co-administration.

8.3.6 Conclusions: *In-Vivo* Equivalence of Salamol Without and With Spacer

The relative lung deposition of Salamol with VOL and AERO was significantly greater than that obtained with the MDI alone. The two delivery systems (MDI Vs MDI+SP) were bio-inequivalent and therefore can have clinical implications. However, the USAL0.5 was bioequivalent between the two spacer treatment methods despite their significantly different volumes.

The total systemic bioavailability (USAL24NC) of the two delivery systems (three treatment methods) was equivalent and statistically similar. It is therefore highly likely that these will have similar salbutamol related systemic effects. Further, USAL24NC between the two spacer treatment methods is bioequivalent and statistically similar which may indicate a similar pattern of systemic effects.

Although FPD is suggestive of *in-vitro* equivalence of the three treatment methods, this could not be observed with *in-vivo* relative lung deposition. Hence, caution should be exercised in relating *in-vitro* and *in-vivo* results.

The relative lung deposition (USAL0.5), total systemic bioavailability (USAL24) and bioavailability of active salbutamol (USAL24Pre) were *in-vivo* equivalent and statistically similar between the two Salamol spacer treatment methods in both legs of the study. These findings suggest that both VOL and AERO should provide equally effective and similarly tolerated relief from bronchoconstriction. Whether one of these spacers could be exchanged for the other with Salamol would be at the discretion of the prescriber and the patient.

The co-administration of activated charcoal clearly differentiated between the amount of Salamol that was deposited in the lungs and that which was absorbed through the GIT after swallowing.

The results of this study should not be interpolated to either other salbutamol MDIs or spacers.

9 Chapter 9: Summary, General Conclusions and Future work

9.1 Summary and General Conclusions

The *in-vitro* dose delivery characteristics of salbutamol HFA MDIs, Ventolin Evohaler (Evo), Airomir (Airo) and Salamol (Sala) have been assessed and complemented with *in-vivo* evaluation in humans. These studies included comparisons of the MDIs with each other and between the MDI alone and when attached to the spacer. The *in-vitro* studies were carried out using Andersen Cascade Impactor (ACI) (BP, 2005; USP28-NF23, 2005; Ph. Eur., 2011) while urinary pharmacokinetic (PK) method of Hindle and Chrystyn (1992) was applied for *in-vivo* assessment. The PK studies were also complemented by charcoal block studies (EMA, 2009).

9.1.1 Salbutamol MDIs

Evo, Airo and Sala had *in-vitro* equivalent and statistically similar emitted dose (Table 5.2.7). However, the FPD (and FPM) was only *in-vitro* equivalent and statistically similar between Airo and Sala. Further, Induction Port (IP) deposition was *in-vitro* inequivalent between these MDIs, while only being marginally similar between Evo and Sala ($p = 0.055$). This was expected. Evo is formulated in HFA134a propellant only, has larger metered dose volume and actuator orifice diameter (Table 2.2.1); hence releases relatively more ballistic spray than the other MDIs (Barry and O'Calaghan, 1997; Gabrio et al. 1999; Brambilla et al., 2011; Hautmann et al., 2013; Johnson et al., 2016; Kunda et al., 2017). Airo and Sala contain ethanol which slows down their spray speed and evaporation of the propellant (Stein and Myrdal, 2006). It is therefore clear that their differences in the dose emission characteristics have origin in formulation and device design.

Although statistically similar, the relative lung deposition (USAL0.5) was not *in-vivo* equivalent amongst the MDIs in both parts of the study (without and with charcoal block). While statistically similar total systemic bioavailability (USAL24) was also observed between them, only paired comparison of Evo Vs Sala and Airo Vs Sala were *in-vivo* equivalent to Parameswaran (1999) limits.

In Part 2 Study, with concomitant charcoal intake, USAL0.5C reproduced similar trend as that of USAL0.5NC, which re-affirms that urinary excretion of salbutamol in the first half an hour post-inhalation is derived from the lung deposition (Hindle and Chrystyn, 1992). Nevertheless, total systemic bioavailability (USAL24C) was *in-vivo* inequivalent with significant statistical difference. This was anticipated since GIT absorption of the

swallowed dose was prevented by charcoal. The co-administration of charcoal clearly differentiated between the systemic salbutamol derived from the lungs and absorbed from the GIT (Table 5.3.9).

The results of the urinary PK study suggest that Evo, Airo and Sala were not *in-vivo* equivalent in healthy subjects. Hence, caution should be exercised if a change of salbutamol MDI brand is desired. Indeed, MHRA (Chrystyn and Price, 2009) and health authorities (NHS Gloucestershire, 2017) have instructed physicians to prescribe MDIs by brand names.

9.1.2 Salbutamol MDI with spacer

A general tendency of significantly greater lung deposition (USAL0.5) with spacer use was observed irrespective of the MDI and spacer brands. However, this increase was not seen with *in-vitro* respirable dose (FPD). Differences in FPD of the two treatment methods (MDI and MDI+SP) were not significant and, instead, were *in-vitro* equivalent. Although unexpected and is in conflict with previous findings for Airomir (Barry and O'Callaghan, 1997; Ross and Gabrio, 1999; Mitchell et al., 1999), this phenomenon is understandable since *in-vitro* findings are not always replicated in humans (Dompeling et al., 2001; Barben et al., 2003; Dubus et al., 2003). Further, in contrast to dynamic human respiratory tract physiology and anatomy, the *in-vitro* experiments are conducted under controlled conditions in rigid systems. The constant flow in ACI may have masked the differences between the MDI alone and with the spacer (Table 6.2.9, Table 7.2.9 & Table 8.2.9). The airflow effectively carried away the emitted dose of the two treatment methods into ACI. This is in line with the findings for Ventolin Evohaler (Cripps et al., 2000) and ProAir HFA (von Hollen et al., 2011a & b; Hatley et al., 2014). Nevertheless, the significant differences of note between the two aerosol delivery systems (MDI Vs MDI+SP) were the composition of delivered dose containing the relative proportions of FPD [%FPF (%TDD)] and the deposition in the IP. This was predictable as spacer retains a large proportion of the emitted dose containing large particles, possessing greater impaction force, ballistic speed and larger spray cone while simultaneously providing space to them to evaporate into finer particles and slowing their speed (Terzano, 2001; Hess, 2008; Nikander et al., 2014). Consequently, the delivered dose contained particles mainly in the size range of FPD. On the contrary, when the MDI is used alone, a large proportion of the emitted dose is deposited in IP.

There is less space to slow down the emitted dose and therefore a large proportion of the ballistic dose deposits in IP. These *in-vitro* findings indicate the role of spacer in significantly reducing the IP deposition. This benefit was obvious in humans where the lung deposition was significantly greater with the spacer than without it. This is partly due to the more effective and consistent inhalation manoeuvre with the spacer use and partly due to the absence of reflexive cessation observed with the impaction of emitted dose on the throat from an MDI.

The only out of trend *in-vitro* result observed was between Evo Vs Evo+AERO where FPD of Evo with AERO was significantly lower than the MDI. This is more likely due to the ballistic emitted dose with large spray cone, eventually resulting in larger spacer deposition (52% of TED) and smaller TDD. Cripps et al. (2000) also reported similar findings for Evo with Babyhaler and VOL. Further, the current results indicate that the volume of the spacer may not have played a significant role. This finding is in conflict with the reported significant increase in FPD with increase in spacer volume for Airomir (Mitchell et al., 1999; Ross and Gabrio, 1999), which however, did not reach significance for Evo (Cripps et al., 2000; Hall et al., 2011). Although spacer and MDI specific differences are often observed (Barry and O'Calaghan, 1997; Ross and Gabrio, 1999; Dubus et al., 2001), as with Evo+AERO in the current study (Table 6.2.4, Table 6.3.9 and Table 6.3.10), it is highly unlikely that small differences in FPD between the two treatment methods (MDI and MDI+SP) may be of clinical significance.

Intriguingly, Salamol MDI alone had greater FPD than with VOL and AERO (Table 8.2.4). This suggests that Salamol alone maybe a better choice than the spacer treatment method. Also, albeit being lower by only <2 µg, FPD of Airomir was statistically similar and *in-vitro* equivalent to that with AERO (Table 7.2.8 & Table 7.2.9). However, *in-vivo* results do not support these assessments since spacers had significantly greater lung deposition than the MDI in healthy humans (Table 8.3.1, Table 8.3.5, Table 8.3.6, Table 7.3.1 & Table 7.3.5). Given that patients have reduced airway calibre (Lipworth and Clark, 1997) and poor inhalation technique (Laube et al., 2011; Lavorini, 2013, 2014; Lavorini et al., 2014), the observed increase in lung deposition with spacer treatment methods may be beneficial.

The significant decrease in the *in-vitro* delivered dose with the spacer as compared to the MDI did not reflect on total systemic bioavailability (USAL24NC). USAL24NC of

the MDIs was *in-vivo* equivalent and statistically similar to the spacer treatment methods.

Interestingly, this was also observed between the MDI alone treatment methods, except that Evo was not *in-vivo* equivalent to Airo.

In contrast, the USAL24C increased significantly with the spacer treatment methods as compared to the MDI. This is more likely due to the greater lung deposition with the spacer. Interestingly, with AERO, the treatment methods of Airo and Sala showed *in-vivo* equivalent USAL24C to their respective MDIs. This out of trend finding highlights that each spacer influences the dose delivery of a given salbutamol MDI differently (Barry and O'Calaghan, 1997; Ross and Gabrio, 1999; Dubus et al., 2001). Further, these results re-inforce that outcomes of one spacer study cannot be extrapolated to other spacers and salbutamol MDIs or an MDI of a different drug (Mitchell and Nagel, 2007; Laube et al., 2011; Nikander et al., 2014).

The comparative *in-vitro* and *in-vivo* results of salbutamol MDIs without and with spacers do not reflect on each other. The statistical similarity of FPD is not reproduced in significantly increased lung deposition. On the other hand, the significant decrease in *in-vitro* delivered dose did not represent the *in-vivo* statistical similarity of total systemic bioavailability in Part 1 Study and to latter's significant increase in Part 2 Study. These results clearly show that *in-vitro* results may not always mimic the *in-vivo* findings. Hence, caution should be exercised in making decisions based on the *in-vitro* data only.

Overall, the results highlight that the MDI delivers FPD inefficiently as compared to the spacer treatment methods as evident from significantly greater FPF (%TDD) (Table 6.2.6 & Table 6.2.9, Table 7.2.6 & Table 7.2.9, Table 8.2.6 & Table 8.2.9). This is clearly reflected in greater lung deposition (Table 6.3.1 & Table 6.3.2, Table 7.3.1 & Table 7.3.5, Table 8.3.1 & Table 8.3.6). The spacer therefore removes non-respirable dose that otherwise would deposit in the throat.

9.2 Future work

The current work assessed *in-vitro* particle size characteristics of salbutamol HFA MDIs as stipulated by the regulatory authorities which is a standard practice in the pharmaceutical companies. Therefore, this work can be extended to other areas to further explore dose delivery characteristics of salbutamol HFA MDIs and to identify

their link to the *in-vivo* studies in humans. Hence, *in-vitro* research using ACI or NGI can include the following areas:

1. The current *in-vivo* studies in humans revealed prolonged excretion of salbutamol post-inhalation, both as active and metabolised moieties (Silkstone et al., 2000, 2002a). This was reproduced in charcoal block studies indicating that the absorption of salbutamol from the lungs may have been taking place over extended time period. Hence, it would be interesting to explore and differentiate *in-vitro* whether this prolonged excretion is due to the dissolution of inhaled salbutamol particles or is an intrinsic characteristic of the molecule. The dynamics of total delivered (TDD) and respirable (FPD) doses, collected on filters, can be explored with *in-vitro* dissolution studies (May et al., 2012, 2014, 2015; Riley et al., 2012; Son et al., 2010). The dose-laden filters are then to undergo dissolution to generate profiles. The following methods could be adopted to collect doses:
    a) Filtration unit for TDD.
    b) Filters placed in glass twin impinger and/or abbreviated impactors (AIM) for FPD and non-respirable dose. Modifications in AIM can also be used for stage group segregation.
    c) Filter on each stage of the impactor (ACI or NGI) for Individual stage depositions. This can pose HPLC method sensitivity challenges.
2. The extent of deposition in the throat can affect the FPD (Golshahi and Finlay, 2012; Borgström et al., 2006). USP throat has been shown to have different deposition patterns than those of mouth-throat models and casts (Zhang et al., 2007; Zhou et al., 2011). Hence, it would be interesting to discover any impact on FPD and stage group deposition of salbutamol MDIs. *In-vitro* studies using Alberta Idealized Throats (Copley et al., 2011; Copley, 2015), throat geometry models and replicas (Fadl et al., 2007; Zhou et al., 2011; Olsson et al., 2013) can provide useful insight into APSD profiles that could then be compared with *in-vivo* lung deposition in humans using urinary PK studies.
3. The flow rate through the impactors can also affect the inhaled dose (von Hollen et al., 2013; Slator et al., 2014; Liu et al., 2017). Hence these throat models should be compared using the following flow rates
    a) standard flow rate 28.3 (ACI) or 30 (NGI) L/min
    b) simulated flow rate
    c) breathing simulation

d) respiratory profiles of volunteers / patients (Chrystyn et al., 2015)

4. Extended comparison should be carried out to include newly marketed and existing spacers. Spacer studies should be conducted on the above mentioned flow rates (No. 3). Tidal breathing patterns should also be explored with the spacers. Moreover, these studies can also include spacer with mask.
5. The experimental conditions of the current *in-vitro* work reflect on the adult population. This work can, therefore, be expanded to conditions that mimic child population. Hence, flow rate of 15 L/min can be used with all the experiments listed above [Nos. 2 & 3].
6. *In-vitro* and *in-vivo* studies should be carried out to other salbutamol MDIs (Asmavent, Asthalin, AirSalb, Apo-salbutamol; Respigen, Ratio-salbutamol) with and without spacer.
7. *In-vitro* and *in-vivo* studies should be conducted to compare breath-actuated salbutamol MDIs (Airomir Autohaler, Salamol Easi-Breathe) with:
    a) their standard versions without and with spacer
    b) other standard MDIs without and with spacer.
8. Further expansion of studies in Nos. 6 & 7 could include those enumerated in Nos. 1-5.
9. Differing CQAs results have been observed for salbutamol MDIs across regions (Table 2.5.1, Table 2.5.2 & Table 2.5.3). It would be interesting to compare these MDIs marketed in different regions if manufactured at different sites. This is to identify if these differences are related to laboratories or multiple manufacturing sites or are due to batch to batch variability.

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Appendices

Appendices Chapter 5

Appendix 5.2.3.1: APSD of two puffs (200 µg) of Ventolin Evohaler MDI obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	4.85	4.71	5.80	4.53	4.97	4.97	0.49	9.91	4.85
MDI Actuator	30.95	37.01	37.55	37.92	37.55	36.20	2.95	8.15	37.55
ACI Throat	96.98	88.47	89.33	77.03	92.08	88.78	7.36	8.29	89.33
ACI S-0	1.84	2.28	2.18	1.92	2.05	2.06	0.18	8.83	2.05
ACI S-1	3.21	2.60	3.03	2.39	2.56	2.76	0.35	12.52	2.60
ACI S-2	3.72	4.94	5.21	4.61	4.62	4.62	0.56	12.15	4.62
ACI S-3	14.91	21.09	19.05	18.48	18.38	18.38	2.23	12.13	18.48
ACI S-4	30.64	35.63	36.76	36.08	36.76	35.17	2.58	7.33	36.08
ACI S-5	16.56	20.93	21.08	20.03	21.08	19.94	1.94	9.72	20.93
ACI S-6	3.50	3.24	3.80	3.05	3.40	3.40	0.28	8.29	3.40
ACI S-7	0.70	0.54	0.73	0.55	0.63	0.63	0.08	13.23	0.63
ACI Filter	1.14	0.69	1.00	0.77	0.90	0.90	0.18	19.94	0.90
Total Recovery (µg)	208.99	222.14	225.51	207.36	224.98	217.80	8.89	4.08	222.14
% Recovery[a]	104.50	111.07	112.76	103.68	112.49	108.90	4.45	4.08	111.07
Mass Balance[b] (µg)	204.14	217.43	219.71	202.83	220.01	212.82	8.59	4.04	217.43
% Recovery	102.07	108.71	109.85	101.42	110.00	106.41	4.30	4.04	108.71

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 5.2.3.2: APSD of two puffs (200 µg) of Airomir MDI obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	22.90	20.49	19.58	15.42	19.58	19.60	2.70	13.77	19.58
MDI Actuator	19.07	18.93	22.82	21.36	22.82	21.00	1.92	9.13	21.36
ACI Throat	73.15	68.95	68.11	70.08	68.11	69.68	2.10	3.02	68.95
ACI S-0	2.73	2.79	4.36	3.01	4.36	3.45	0.84	24.29	3.01
ACI S-1	2.53	3.15	4.70	5.00	4.70	4.01	1.10	27.48	4.70
ACI S-2	3.76	5.51	7.07	5.58	7.07	5.80	1.37	23.67	5.58
ACI S-3	20.01	25.65	25.22	29.52	25.22	25.12	3.38	13.47	25.22
ACI S-4	38.55	35.18	34.16	34.53	36.08	35.70	1.75	4.90	35.18
ACI S-5	22.56	21.05	21.46	18.70	21.46	21.04	1.43	6.77	21.46
ACI S-6	5.22	5.21	5.54	4.43	5.54	5.19	0.45	8.72	5.22
ACI S-7	2.05	2.08	2.02	2.08	2.02	2.05	0.03	1.46	2.05
ACI Filter	2.72	2.65	2.14	1.74	2.14	2.28	0.41	17.82	2.14
Total Recovery (µg)	215.25	211.64	217.17	211.46	219.08	214.92	3.36	1.56	215.25
% Recovery[a]	107.62	105.82	108.59	105.73	109.54	107.46	1.68	1.56	107.62
Mass Balance[b] (µg)	192.35	191.15	197.59	196.04	199.50	195.32	3.51	1.80	196.04
% Recovery	96.17	95.57	98.79	98.02	99.75	97.66	1.76	1.80	98.02

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff

b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 5.2.3.3: APSD of two puffs (200 µg) of Salamol MDI obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	23.24	20.02	17.35	20.56	19.04	20.04	2.17	10.80	20.02
MDI Actuator	34.83	33.25	28.29	22.13	25.50	28.80	5.29	18.36	28.29
ACI Throat	82.55	79.64	80.56	82.52	75.07	80.07	3.07	3.83	80.56
ACI S-0	1.81	2.56	3.43	2.70	3.05	2.71	0.61	22.37	2.70
ACI S-1	2.80	3.57	4.14	3.42	4.93	3.77	0.80	21.32	3.57
ACI S-2	3.49	3.82	4.06	4.79	4.18	4.07	0.48	11.88	4.06
ACI S-3	18.15	22.48	20.23	18.13	15.74	18.95	2.53	13.38	18.15
ACI S-4	35.37	36.89	28.70	37.75	40.25	35.79	4.34	12.13	36.89
ACI S-5	23.06	24.85	18.72	23.94	29.29	23.97	3.79	15.81	23.94
ACI S-6	4.75	5.72	6.41	4.99	7.10	5.80	0.98	16.92	5.72
ACI S-7	1.83	2.11	2.96	1.91	2.66	2.30	0.49	21.51	2.11
ACI Filter	2.10	2.81	3.82	2.67	1.63	2.60	0.82	31.65	2.67
Total Recovery (µg)	233.97	237.73	218.67	225.51	228.45	228.87	7.42	3.24	228.45
% Recovery[a]	116.99	118.87	109.34	112.75	114.22	114.43	3.71	3.24	114.22
Mass Balance[b] (µg)	210.73	217.71	201.32	204.94	209.41	208.82	6.21	2.98	209.41
% Recovery	105.37	108.86	100.66	102.47	104.71	104.41	3.11	2.98	104.71

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff

b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 5.3.4.1: Volunteer's demographic data

Volunteer Id	Sex	Age	Heights	Weight	BMI
	M/F	(years)	(m)	(Kg)	(Kg/m^2)
1	F	27	1.70	72	24.91
2	M	38	1.73	81	27.06
3	F	31	1.58	50	20.03
4	M	38	1.68	69	24.45
5	F	27	1.65	61	22.41
6	F	25	1.65	68	24.98
7	M	48	1.82	82	24.76
8	F	26	1.57	55	22.31
9	F	25	1.62	48	18.29
10	M	39	1.64	54	20.08
11	M	33	1.79	71	22.16
12	F	23	1.68	65	23.03
13	M	25	1.70	67	23.18
Mean		31.15	1.68	64.85	22.90
SD		7.55	0.07	10.84	2.42
Median		27	1.68	67	10.57

M = male; F = female; BMI = Body Mass Index

Appendix 5.3.4.2: Ventolin Evohaler Part 1 Study (without charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components				TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**TDD**	**TDD %ND**	
1	4.0	110.8	45.0	108.6	63.6	4.1	30.7	169.3	84.6	58.4
2	6.6	92.9	32.3	88.6	56.3	4.3	42.3	157.7	78.9	64.9
3	4.8	74.4	41.8	71.8	29.9	4.7	39.9	160.1	80.1	85.8
4	4.2	114.6	64.4	111.7	47.3	6.0	47.0	153.0	76.5	38.4
5	7.1	97.1	72.6	87.8	15.2	5.8	34.3	165.7	82.8	68.6
6	2.5	118.1	94.5	114.8	20.3	5.0	34.0	166.0	83.0	47.9
7	8.0	103.2	55.7	96.4	40.7	5.4	33.3	166.7	83.3	63.4
8	8.4	79.6	33.0	75.7	42.8	7.0	46.4	153.6	76.8	74.0
9	6.9	107.4	77.2	97.7	20.5	3.9	39.8	160.2	80.1	52.8
10	4.1	86.3	49.9	81.3	31.4	4.5	38.2	161.8	80.9	75.6
11	4.3	116.9	63.2	108.9	45.8	5.7	44.3	155.7	77.9	38.8
12	5.6	123.7	73.1	118.7	45.6	3.9	36.7	163.3	81.7	39.6
13	7.8	78.5	56.2	72.6	16.4	3.9	45.5	154.5	77.2	76.0
Mean	5.7	100.26	58.4	95.0	36.6	4.94	39.41	160.6	80.3	60.3
SD	1.9	16.72	18.3	16.6	15.6	0.97	5.43	5.4	2.7	15.8
RSD	32.5	16.68	31.3	17.5	42.6	19.60	13.77	3.4	3.4	26.2

[a] TRD

Appendix 5.3.4.3: Airomir Part 1 Study (without charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components				TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**TDD**	**TDD %ND**	
1	9.7	95.0	53.9	85.4	31.4	15.1	21.6	178.4	89.2	83.4
2	4.9	77.5	44.9	72.5	27.7	17.4	24.8	175.2	87.6	97.7
3	5.5	68.7	44.8	63.3	18.5	18.9	25.5	174.5	87.2	105.7
4	6.2	67.9	43.8	61.7	17.9	17.1	23.3	176.7	88.4	108.8
5	6.2	75.4	46.3	69.2	22.9	13.6	18.9	181.1	90.5	105.7
6	7.8	72.9	32.8	65.1	32.3	12.9	23.6	176.4	88.2	103.5
7	5.7	96.6	59.2	90.9	31.7	13.9	25.4	174.6	87.3	78.0
8	8.6	97.6	55.9	89.1	33.1	18.2	23.4	176.6	88.3	79.0
9	10.0	95.1	56.1	85.1	29.0	14.6	26.7	173.3	86.6	78.2
10	5.3	96.6	48.0	91.3	43.3	16.7	22.6	177.4	88.7	80.8
11	5.1	100.0	49.6	94.9	45.3	13.1	24.0	176.0	88.0	76.0
12	6.6	66.5	37.8	59.9	22.1	13.7	25.0	175.0	87.5	108.5
13	10.9	84.9	42.2	74.1	31.8	16.2	19.6	180.4	90.2	95.5
Mean	7.11	84.22	47.36	77.11	29.76	15.49	23.41	176.59	88.30	92.37
SD	2.05	13.06	7.61	12.74	8.34	2.04	2.29	2.29	1.15	13.29
RSD	28.88	15.50	16.06	16.52	28.02	13.17	9.80	1.30	1.30	14.39

[a] TRD

Appendix 5.3.4.4: Salamol Part 1 Study (without charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components				TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**TDD**	**TDD %ND**	
1	6.7	87.8	46.7	81.2	34.4	16.8	23.9	176.1	88.0	88.2
2	6.1	102.6	57.6	96.4	38.8	15.7	28.3	171.7	85.9	69.2
3	5.9	75.7	33.6	69.8	36.1	14.9	26.0	174.0	87.0	98.3
4	10.7	101.1	46.9	90.5	43.6	18.1	28.1	171.9	86.0	70.8
5	6.9	62.4	14.9	55.5	40.6	20.3	26.4	173.6	86.8	111.2
6	1.9	81.8	37.1	79.8	42.7	17.4	19.6	180.4	90.2	98.6
7	5.6	111.2	42.2	105.6	63.4	17.6	29.7	170.3	85.2	59.1
8	5.0	128.1	47.8	123.0	75.2	15.6	26.0	174.0	87.0	46.0
9	12.9	124.6	56.7	111.6	55.0	22.8	27.0	173.0	86.5	48.4
10	7.0	81.3	36.3	74.3	37.9	15.2	29.3	170.7	85.3	89.3
11	2.1	86.7	52.4	84.6	32.2	18.6	19.9	180.1	90.0	93.4
12	5.2	80.0	42.6	74.8	32.2	15.7	30.0	170.0	85.0	90.0
13	11.0	103.3	42.7	92.3	49.6	28.9	21.2	178.8	89.4	75.5
Mean	6.70	94.35	42.89	87.65	44.75	18.29	25.80	174.20	87.10	79.85
SD	3.22	19.44	11.17	18.41	12.92	3.89	3.61	3.61	1.80	20.21
RSD	48.11	20.60	26.05	21.00	28.86	21.26	13.99	2.07	2.07	25.31

[a] TRD

Appendix 5.3.4.5: Ventolin Evohaler Part 2 Study (with charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components				TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**TDD**	**TDD %ND**	
1	2.2	22.5	13.6	20.3	6.7	5.4	38.3	161.7	80.9	139.2
2	4.3	31.9	15.6	27.7	12.0	4.7	42.6	157.4	78.7	125.5
3	2.6	34.2	16.3	31.6	15.3	4.9	45.1	154.9	77.4	120.7
4	2.8	32.0	20.6	29.2	8.6	5.3	44.8	155.2	77.6	123.3
5	9.3	36.9	21.4	27.6	6.2	4.7	35.1	164.9	82.4	128.0
6	3.4	18.7	10.0	15.3	5.3	5.3	37.1	162.9	81.4	144.2
7	6.8	30.7	12.6	23.9	11.3	4.9	37.8	162.2	81.1	131.4
8	3.8	30.2	8.0	26.4	18.4	5.2	38.8	161.2	80.6	131.0
9	9.7	33.1	7.6	23.4	15.8	4.8	37.8	162.2	81.1	129.1
10	5.0	25.8	15.2	20.8	5.5	5.8	38.5	161.5	80.8	135.7
11	8.0	31.8	6.8	23.8	17.0	6.3	45.9	154.1	77.0	122.3
12	5.0	34.8	23.4	29.8	6.4	4.7	42.0	158.0	79.0	123.2
13	5.9	24.6	15.0	18.8	3.7	4.5	46.4	153.6	76.8	129.0
Mean	5.29	29.79	14.3	24.5	10.2	5.11	40.78	159.22	79.61	129.43
SD	2.50	5.31	5.3	4.8	5.1	0.51	3.83	3.83	1.91	6.96
RSD	47.28	17.83	37.2	19.5	49.7	9.88	9.38	2.40	2.40	5.38

[a] TRD

Appendix 5.3.4.6: Airomir Part 2 Study (with charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components				TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**TDD**	**TDD %ND**	
1	6.6	55.5	36.1	48.9	12.8	16.5	25.2	174.8	87.4	119.3
2	5.5	36.2	18.8	30.7	11.9	15.6	19.5	180.5	90.3	144.3
3	4.6	39.0	21.1	34.4	13.3	14.6	26.8	173.2	86.6	134.2
4	8.4	55.6	36.0	47.3	11.3	16.6	23.3	176.7	88.4	121.1
5	4.7	29.0	16.0	24.3	8.3	13.1	18.0	182.0	91.0	153.0
6	5.8	29.3	18.4	23.5	5.1	13.6	24.9	175.1	87.6	145.8
7	8.2	62.2	42.9	54.0	11.1	14.0	25.6	174.4	87.2	112.1
8	6.8	57.2	29.0	50.3	21.3	16.1	26.4	173.6	86.8	116.4
9	9.2	66.6	42.8	57.3	14.6	15.8	21.9	178.1	89.0	111.5
10	5.2	47.1	26.4	41.9	15.5	14.8	24.1	175.9	88.0	128.8
11	4.5	51.2	35.4	46.7	11.3	14.6	26.7	173.3	86.6	122.1
12	5.1	50.1	31.8	45.0	13.2	14.2	25.9	174.1	87.0	123.9
13	11.9	51.6	30.8	39.6	8.9	13.7	25.1	174.9	87.4	123.3
Mean	6.67	48.52	29.65	41.84	12.19	14.87	24.11	175.89	87.95	127.38
SD	2.22	11.90	9.04	10.80	3.88	1.14	2.76	2.76	1.38	13.21
RSD	33.21	24.52	30.47	25.81	31.86	7.67	11.46	1.57	1.57	10.37

[a] TRD

Appendix 5.3.4.7: Salamol Part 2 Study (with charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components				TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**TDD**	**TDD %ND**	
1	6.2	25.9	12.6	19.7	7.2	18.2	19.7	180.3	90.1	154.4
2	4.1	37.9	18.0	33.8	15.8	12.8	25.6	174.4	87.2	136.5
3	2.8	48.5	20.2	45.6	25.4	11.8	26.6	173.4	86.7	125.0
4	11.8	69.1	32.5	57.3	24.8	12.7	29.6	170.4	85.2	101.4
5	8.5	61.5	22.3	53.0	30.7	12.0	30.4	169.6	84.8	108.0
6	7.9	42.5	25.1	34.5	9.4	25.3	31.0	169.0	84.5	126.5
7	6.3	62.7	38.1	56.4	18.4	13.4	31.4	168.6	84.3	105.9
8	5.6	48.4	32.6	42.8	10.2	11.8	25.5	174.5	87.2	126.1
9	15.5	30.8	8.7	15.4	6.7	12.2	26.8	173.2	86.6	142.4
10	5.5	51.4	20.7	45.9	25.2	13.3	27.1	172.9	86.5	121.5
11	4.8	52.8	42.4	48.0	5.7	25.5	19.3	180.7	90.3	127.9
12	8.2	62.2	33.8	54.0	20.3	13.6	27.9	172.1	86.0	109.8
13	6.6	58.6	47.0	52.0	5.0	26.1	22.7	177.3	88.6	118.7
Mean	7.20	50.17	27.23	42.96	15.74	16.04	26.43	173.57	86.78	123.40
SD	3.36	13.06	11.55	13.50	8.93	5.70	3.92	3.92	1.96	15.18
RSD	46.60	26.03	42.43	31.43	56.77	35.55	14.83	2.26	2.26	12.30

[a] TRD

Appendix 6.2.6.1: APSD of two puffs (200 µg) of Ventolin Evohaler + Volumatic obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	5.43	6.14	5.15	6.99	7.11	6.17	0.89	14.36	6.14
MDI Actuator	22.52	21.28	23.79	24.84	23.28	23.14	1.34	5.79	23.28
Spacer	82.59	78.34	66.36	74.77	72.40	74.89	6.13	8.18	74.77
ACI Throat	6.44	7.15	5.97	2.26	5.71	5.51	1.90	34.42	5.97
ACI S-0	1.65	1.95	1.38	2.05	2.63	1.93	0.47	24.36	1.95
ACI S-1	4.51	3.22	4.51	3.92	3.05	3.84	0.69	18.00	3.92
ACI S-2	6.14	3.75	7.26	5.16	5.32	5.53	1.30	23.45	5.32
ACI S-3	15.18	18.25	20.50	22.84	23.62	20.08	3.45	17.20	20.50
ACI S-4	32.23	33.45	30.56	34.45	35.42	33.22	1.90	5.72	33.45
ACI S-5	17.29	18.95	19.80	21.30	17.58	18.98	1.65	8.68	18.95
ACI S-6	5.25	4.14	3.24	2.96	4.32	3.98	0.91	22.95	4.14
ACI S-7	0.82	0.97	1.05	0.72	0.91	0.89	0.13	14.38	0.91
ACI Filter	0.95	0.82	1.15	0.65	1.03	0.92	0.19	20.96	0.95
Total Recovery (µg)	201.00	198.42	190.73	202.91	202.38	199.09	4.99	2.50	201.00
% Recovery[a]	100.50	99.21	95.36	101.45	101.19	99.54	2.49	2.50	100.50
Mass Balance[b] (µg)	195.57	192.27	185.57	195.92	195.27	192.92	4.36	2.26	195.27
% Recovery	97.79	96.14	92.79	97.96	97.63	96.46	2.18	2.26	97.63

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 6.2.6.2: APSD of two puffs (200 µg) of Ventolin Evohaler + AeroChamber Plus obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	6.73	5.15	7.18	4.09	5.63	5.76	1.24	21.52	5.63
MDI Actuator	22.90	24.66	25.30	26.18	21.56	24.12	1.87	7.75	24.66
Spacer	86.55	96.56	98.99	87.41	83.61	90.62	6.73	7.43	87.41
ACI Throat	4.79	5.65	3.86	6.62	4.17	5.02	1.13	22.45	4.79
ACI S-0	2.11	1.45	1.92	2.99	3.05	2.30	0.70	30.24	2.11
ACI S-1	5.14	3.95	3.33	4.62	5.05	4.42	0.77	17.40	4.62
ACI S-2	7.25	4.92	5.69	6.61	6.12	6.12	0.89	14.49	6.12
ACI S-3	21.33	17.63	15.26	20.48	19.66	18.87	2.44	12.94	19.66
ACI S-4	25.23	31.13	28.66	30.52	27.87	28.68	2.34	8.17	28.66
ACI S-5	15.11	13.54	17.05	16.25	14.84	15.36	1.35	8.79	15.11
ACI S-6	3.62	2.82	1.85	2.22	2.88	2.68	0.68	25.35	2.82
ACI S-7	1.44	0.82	0.77	0.69	0.84	0.91	0.30	32.98	0.82
ACI Filter	1.26	0.94	0.82	0.87	0.98	0.97	0.17	17.60	0.94
Total Recovery (µg)	203.46	209.22	210.67	209.55	196.26	205.83	6.04	2.94	209.22
% Recovery[a]	101.73	104.61	105.34	104.78	98.13	102.92	3.02	2.94	104.61
Mass Balance[b] (µg)	196.72	204.07	203.50	205.46	190.63	200.08	6.27	3.13	203.50
% Recovery	98.36	102.03	101.75	102.73	95.32	100.04	3.13	3.13	101.75

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 6.2.6.3: APSD of two puffs (200 µg) of Ventolin Evohaler + Able obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	8.69	5.00	4.23	7.61	8.16	6.74	1.99	29.58	7.61
MDI Actuator	17.29	22.43	18.21	23.36	18.82	20.02	2.70	13.48	18.82
Spacer	92.59	86.87	91.43	84.97	82.10	87.59	4.40	5.02	86.87
ACI Throat	4.12	4.92	6.45	4.50	5.64	5.13	0.93	18.15	4.92
ACI S-0	2.19	2.80	2.46	2.46	1.73	2.33	0.40	17.03	2.46
ACI S-1	4.12	4.52	3.84	4.73	5.20	4.48	0.53	11.81	4.52
ACI S-2	5.47	7.53	5.39	6.43	6.64	6.29	0.89	14.12	6.43
ACI S-3	16.18	23.63	20.22	16.65	22.58	19.85	3.38	17.00	20.22
ACI S-4	31.16	22.93	23.53	33.11	28.92	27.93	4.55	16.28	28.92
ACI S-5	14.62	16.73	20.07	16.39	18.84	17.33	2.14	12.36	16.73
ACI S-6	6.07	6.13	3.73	5.02	4.23	5.04	1.08	21.34	5.02
ACI S-7	2.10	2.27	1.50	1.71	1.89	1.89	0.31	16.17	1.89
ACI Filter	1.76	1.89	2.36	2.13	1.73	1.97	0.27	13.53	1.89
Total Recovery (µg)	206.35	207.66	203.41	209.06	206.48	206.59	2.09	1.01	206.48
% Recovery[a]	103.18	103.83	101.71	104.53	103.24	103.30	1.04	1.01	103.24
Mass Balance[b] (µg)	197.67	202.67	199.18	201.46	198.32	199.86	2.13	1.06	199.18
% Recovery	98.83	101.33	99.59	100.73	99.16	99.93	1.06	1.06	99.59

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 6.3.4.1: Ventolin Evohaler+Volumatic Part 1 Study (without charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	20.0	104.5	54.7	95.7	40.9	4.1	19.2	67.8	180.8	113.0	56.5	8.5
2	4.1	98.8	61.7	92.7	31.0	4.3	19.4	71.7	180.6	108.9	54.4	10.1
3	25.7	107.4	77.0	92.6	15.6	4.7	19.1	67.7	180.9	113.2	56.6	5.7
4	4.8	112.5	65.4	100.7	35.2	6.0	16.4	65.9	183.6	117.7	58.8	5.2
5	24.7	74.8	45.2	64.4	19.2	5.8	24.4	91.8	175.6	83.8	41.9	9.0
6	10.4	91.4	58.8	75.5	16.7	5.0	25.7	77.7	174.3	96.6	48.3	5.2
7	26.4	93.5	68.8	86.0	17.3	5.4	22.3	77.3	177.7	100.4	50.2	6.9
8	19.3	82.4	60.8	76.9	16.1	7.0	25.3	85.5	174.7	89.2	44.6	6.8
9	24.9	102.4	81.9	96.8	14.9	3.9	22.0	68.0	178.0	110.1	55.0	7.7
10	12.1	80.5	44.9	61.0	16.2	4.5	23.4	88.0	176.6	88.6	44.3	8.1
11	10.5	93.8	60.6	83.4	22.7	5.7	23.8	75.2	176.2	101.0	50.5	7.3
12	20.9	114.8	79.9	95.2	15.3	3.9	17.7	62.7	182.3	119.6	59.8	4.8
13	8.9	108.8	57.1	88.0	30.9	3.9	16.5	69.6	183.5	113.9	57.0	5.1
Mean	16.36	97.35	62.84	85.29	22.45	4.94	21.17	74.52	178.83	104.30	52.15	6.95
SD	8.18	12.67	11.76	12.54	8.93	0.97	3.28	9.13	3.28	11.89	5.94	1.69
RSD	49.99	13.01	18.71	14.71	39.75	19.60	15.49	12.25	1.83	11.40	11.40	24.29

[a] TRD

Appendix 6.3.4.2. Ventolin Evohaler+AeroChamber Plus Part 1 Study (without charcoal blockade)

Salbutamol urinary excretion of individual healthy volunteers						Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	23.9	104.4	64.8	80.5	15.6	4.6	17.1	75.4	182.9	107.5	53.8	3.1
2	5.0	101.5	75.1	95.8	20.7	4.5	17.2	76.4	182.8	106.5	53.2	5.0
3	15.6	86.5	48.7	65.6	16.9	5.0	21.1	88.5	178.9	90.4	45.2	3.9
4	5.1	83.8	71.7	80.9	9.2	6.6	25.2	86.0	174.8	88.7	44.4	5.0
5	22.5	105.2	69.2	82.8	13.5	4.5	19.6	71.7	180.4	108.7	54.4	3.5
6	7.4	30.7	17.3	27.3	10.0	6.8	28.6	128.9	171.4	42.5	21.3	11.8
7	24.0	84.5	65.8	71.5	5.7	4.2	20.5	91.5	179.5	87.9	44.0	3.4
8	17.5	119.2	82.4	100.6	18.2	4.2	13.2	64.5	186.8	122.3	61.2	3.2
9	14.1	78.6	52.3	68.2	15.9	5.5	19.9	97.7	180.1	82.5	41.2	3.8
10	9.2	115.5	75.2	97.4	22.2	4.6	14.2	67.0	185.8	118.8	59.4	3.3
11	14.3	74.9	44.9	60.7	15.7	6.2	23.9	95.8	176.1	80.3	40.2	5.5
12	25.2	47.4	27.1	40.9	13.8	4.4	28.5	119.9	171.5	51.6	25.8	4.2
13	8.8	67.7	47.9	62.7	14.8	4.4	25.8	100.9	174.2	73.3	36.6	5.6
Mean	14.82	84.60	57.10	71.90	14.80	5.05	21.14	89.54	178.86	89.31	44.66	4.72
SD	7.39	25.77	19.57	21.48	4.54	0.92	5.03	19.48	5.03	24.09	12.04	2.31
RSD	49.85	30.46	34.28	29.88	30.66	18.29	23.77	21.76	2.81	26.97	26.97	48.98

[a] TRD

Appendix 6.3.4.3: Ventolin Evohaler+Able spacer Part 1 Study (without charcoal blockade)

Salbutamol urinary excretion of individual healthy volunteers						Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	8.4	72.4	46.0	64.1	18.1	5.9	15.8	100.8	184.2	83.4	41.7	11.0
2	13.3	81.7	54.0	68.4	14.4	5.2	21.3	89.8	178.7	88.9	44.5	7.2
3	15.8	98.7	64.2	82.8	18.7	5.1	16.3	80.5	183.7	103.2	51.6	4.5
4	6.2	63.4	42.2	57.2	15.0	4.5	21.9	107.4	178.1	70.6	35.3	7.2
5	21.4	89.9	58.1	68.5	10.4	5.1	16.6	87.8	183.4	95.6	47.8	5.7
6	6.3	53.6	44.1	47.3	3.2	5.9	25.5	110.5	174.5	64.0	32.0	10.4
7	14.9	84.5	53.3	69.5	16.2	5.1	17.4	92.4	182.6	90.2	45.1	5.7
8	20.4	123.2	86.6	102.8	16.2	3.5	13.7	58.5	186.3	127.8	63.9	4.7
9	18.5	102.9	72.1	84.4	12.3	5.1	18.4	73.5	181.6	108.1	54.1	5.2
10	12.0	82.3	65.5	70.3	4.8	6.3	21.2	92.3	178.8	86.5	43.2	4.2
11	17.4	97.4	66.7	80.0	13.3	6.5	20.6	77.8	179.4	101.6	50.8	4.1
12	21.5	127.2	92.4	105.7	13.3	4.7	16.3	52.2	183.7	131.4	65.7	4.2
13	11.5	89.0	59.7	77.5	17.8	5.5	12.3	92.9	187.7	94.8	47.4	5.8
Mean	14.43	89.70	61.91	75.27	13.36	5.25	18.26	85.88	181.74	95.86	47.93	6.16
SD	5.39	20.98	15.27	16.42	4.79	0.78	3.68	17.26	3.68	19.36	9.68	2.28
RSD	37.36	23.39	24.67	21.81	35.84	14.88	20.14	20.10	2.02	20.19	20.19	37.01

[a] TRD

Appendix 6.3.4.4: Ventolin Evohaler+Volumatic Part 2 Study (with charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	8.8	64.5	35.5	55.7	20.2	5.4	18.8	71.1	181.2	110.1	55.0	45.5
2	6.1	49.1	29.6	43.0	13.4	4.7	22.0	73.2	178.0	104.9	52.4	55.8
3	17.9	71.6	42.5	53.8	11.2	4.9	19.8	69.5	180.2	110.7	55.4	39.1
4	11.8	55.9	26.3	44.1	17.8	5.3	24.5	76.9	175.5	98.6	49.3	42.7
5	19.4	84.1	50.1	64.6	14.5	4.7	24.0	71.2	176.0	104.8	52.4	20.8
6	15.9	58.4	25.8	42.4	16.7	5.3	23.1	79.1	176.9	97.8	48.9	39.4
7	17.5	87.3	51.1	69.8	18.8	4.9	20.7	69.8	179.3	109.5	54.8	22.2
8	19.6	94.7	57.8	75.1	17.3	5.2	22.1	68.8	177.9	109.2	54.6	14.5
9	15.6	83.7	38.0	68.2	30.2	4.8	23.6	70.2	176.4	106.1	53.1	22.4
10	9.4	54.0	36.7	44.6	8.0	5.8	25.2	77.3	174.8	97.5	48.8	43.5
11	10.4	50.4	31.3	40.1	8.8	6.3	24.1	75.7	175.9	100.1	50.1	49.7
12	19.6	69.2	36.1	49.6	13.5	4.7	22.9	73.6	177.1	103.5	51.7	34.3
13	20.8	73.1	46.4	52.3	5.9	4.5	25.1	76.3	174.9	98.6	49.3	25.4
Mean	14.83	68.93	39.01	54.10	15.09	5.11	22.76	73.29	177.24	103.96	51.98	35.03
SD	4.92	15.09	9.99	11.80	6.31	0.51	2.02	3.45	2.02	4.99	2.50	12.79
RSD	33.21	21.89	25.61	21.81	41.83	9.88	8.88	4.71	1.14	4.80	4.80	36.52

[a] TRD

Appendix 6.3.4.5: Ventolin Evohaler+AeroChamber Plus Part 2 Study (with charcoal blockade)

Salbutamol urinary excretion of individual healthy volunteers						Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	6.5	43.6	25.4	37.1	11.6	4.2	22.3	96.4	177.7	81.2	40.6	37.7
2	5.7	53.5	31.6	47.8	16.2	4.8	23.2	92.5	176.8	84.3	42.1	30.8
3	20.9	73.4	39.3	52.6	13.2	4.2	23.3	88.0	176.7	88.7	44.4	15.3
4	6.9	36.6	17.7	29.7	11.9	5.9	25.8	98.2	174.2	76.0	38.0	39.5
5	10.7	48.1	32.2	37.4	5.2	5.1	22.1	92.3	177.9	85.6	42.8	37.5
6	8.4	49.6	35.8	41.2	5.4	5.4	23.4	93.1	176.6	83.5	41.7	33.9
7	21.0	81.6	50.7	60.6	9.9	5.5	23.3	83.7	176.7	92.9	46.5	11.3
8	18.6	78.2	45.0	59.7	14.6	5.5	22.4	86.1	177.6	91.5	45.8	13.3
9	19.4	87.1	46.8	67.6	20.9	4.2	20.1	82.5	179.9	97.3	48.7	10.3
10	18.1	75.7	49.6	57.6	8.0	4.3	21.3	84.8	178.7	93.9	47.0	18.2
11	24.9	90.3	54.7	65.5	10.8	5.5	19.1	80.5	180.9	100.4	50.2	10.1
12	9.5	65.0	45.0	55.4	10.4	4.9	21.1	93.3	178.9	85.6	42.8	20.6
13	9.0	57.7	41.6	48.7	7.1	5.0	23.7	95.7	176.3	80.6	40.3	22.9
Mean	13.81	64.65	39.65	50.83	11.18	4.96	22.40	89.78	177.60	87.82	43.91	23.17
SD	6.72	17.64	10.71	11.78	4.42	0.60	1.71	5.81	1.71	7.09	3.54	11.26
RSD	48.69	27.29	27.02	23.17	39.52	12.02	7.64	6.47	0.96	8.07	8.07	48.59

[a] TRD

Appendix 6.3.4.6: Ventolin Evohaler+Able spacer Part 2 Study (with charcoal blockade)

Salbutamol urinary excretion of individual healthy volunteers						Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	5.4	42.0	30.9	36.7	5.8	5.4	21.3	90.1	178.7	88.6	44.3	46.5
2	6.5	38.7	23.2	32.2	9.0	6.5	20.3	104.2	179.7	75.6	37.8	36.8
3	18.8	86.9	49.0	68.1	19.2	4.1	17.4	74.1	182.6	108.5	54.2	21.6
4	15.3	66.2	42.4	50.8	8.4	5.1	22.2	90.1	177.8	87.7	43.8	21.5
5	7.7	39.3	24.0	31.6	7.6	5.8	21.3	89.7	178.7	89.0	44.5	49.7
6	16.4	74.3	42.6	57.9	15.3	4.7	18.8	82.1	181.2	99.1	49.5	24.8
7	11.4	50.8	26.7	39.3	12.6	6.3	19.4	93.1	180.6	87.5	43.7	36.7
8	23.8	93.5	51.9	69.8	17.9	4.2	17.6	76.2	182.4	106.2	53.1	12.7
9	19.2	77.8	34.8	58.6	23.8	6.2	19.1	78.7	180.9	102.2	51.1	24.4
10	16.3	63.3	38.4	47.0	8.5	5.3	19.5	86.5	180.5	94.0	47.0	30.7
11	7.7	33.5	13.0	25.8	12.8	5.6	23.1	98.3	176.9	78.6	39.3	45.1
12	15.4	63.3	40.4	47.9	7.5	5.4	18.7	81.7	181.3	99.6	49.8	36.3
13	18.9	76.0	37.9	57.1	19.2	6.3	19.1	79.5	180.9	101.4	50.7	25.4
Mean	14.06	61.97	35.01	47.91	12.89	5.45	19.83	86.49	180.17	93.68	46.84	31.71
SD	5.78	19.63	11.08	14.12	5.70	0.77	1.72	8.87	1.72	10.21	5.10	11.20
RSD	41.11	31.68	31.64	29.48	44.23	14.10	8.68	10.25	0.95	10.90	10.90	35.33

[a] TRD

Appendix 7.2.6.1: APSD of two puffs (200 µg) of Airomir + AeroChamber Plus obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	14.28	13.48	12.54	15.85	15.40	14.31	1.36	9.49	14.28
MDI Actuator	21.32	18.73	20.40	19.35	18.88	19.74	1.10	5.58	19.35
Spacer	67.50	72.30	70.25	69.65	74.40	70.82	2.63	3.72	70.25
ACI Throat	5.24	4.14	4.44	5.11	5.33	4.85	0.53	10.88	5.11
ACI S-0	2.20	1.80	2.15	2.29	1.94	2.08	0.20	9.66	2.15
ACI S-1	1.40	1.77	1.96	1.81	1.25	1.64	0.30	18.13	1.77
ACI S-2	5.19	6.68	7.24	7.13	6.28	6.50	0.83	12.74	6.68
ACI S-3	18.46	22.23	16.41	19.80	21.48	19.68	2.34	11.89	19.80
ACI S-4	42.01	36.69	37.05	43.90	40.52	40.04	3.13	7.81	40.52
ACI S-5	21.54	26.40	26.32	23.67	18.84	23.36	3.23	13.84	23.67
ACI S-6	7.61	9.24	5.99	6.15	8.30	7.46	1.40	18.71	7.61
ACI S-7	2.62	1.89	2.27	2.13	1.96	2.17	0.29	13.28	2.13
ACI Filter	1.71	2.25	2.43	1.82	1.86	2.01	0.31	15.37	1.86
Total Recovery (µg)	211.08	217.60	209.46	218.68	216.45	214.66	4.12	1.92	216.45
% Recovery[a]	105.54	108.80	104.73	109.34	108.23	107.33	2.06	1.92	108.23
Mass Balance[b] (µg)	196.80	204.12	196.92	202.83	201.05	200.34	3.36	1.68	201.05
% Recovery	98.40	102.06	98.46	101.42	100.53	100.17	1.68	1.68	100.53

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 7.3.4.1: Airomir+AeroChamber Plus Part 1 Study (without charcoal blockade)

Salbutamol urinary excretion of individual healthy volunteers						Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	8.3	70.3	48.9	62.1	13.2	16.0	21.7	78.2	178.3	100.1	50.0	29.8
2	9.6	86.5	52.5	76.9	24.4	17.2	20.6	76.5	179.4	103.0	51.5	16.5
3	13.5	79.3	58.0	65.7	7.8	14.3	19.1	81.1	180.9	99.8	49.9	20.5
4	17.2	94.5	64.1	77.3	13.2	15.7	15.7	76.0	184.3	108.3	54.1	13.8
5	21.2	97.7	69.6	76.5	7.0	13.0	15.3	71.4	184.7	113.4	56.7	15.6
6	20.2	91.6	62.0	71.4	9.4	20.0	19.9	78.2	180.1	101.9	50.9	10.3
7	14.4	77.1	45.7	62.8	17.1	17.3	20.9	79.1	179.1	100.0	50.0	22.9
8	15.7	82.0	57.6	66.3	8.7	14.5	18.4	82.4	181.6	99.1	49.6	17.1
9	17.6	83.1	56.1	65.5	9.4	19.9	19.6	84.3	180.4	96.2	48.1	13.1
10	12.8	80.6	55.0	67.7	12.7	12.7	22.3	86.0	177.7	91.7	45.9	11.2
11	20.9	92.4	63.9	71.5	7.6	17.6	19.0	75.3	181.0	105.8	52.9	13.4
12	12.2	76.7	52.7	64.5	11.8	12.3	21.8	82.4	178.2	95.8	47.9	19.2
13	12.6	79.4	54.3	66.8	12.5	19.9	22.8	84.6	177.2	92.6	46.3	13.2
Mean	15.09	83.93	56.94	68.85	11.90	16.17	19.77	79.65	180.23	100.58	50.29	16.65
SD	4.16	8.07	6.62	5.37	4.74	2.75	2.32	4.28	2.32	6.09	3.04	5.39
RSD	27.60	9.62	11.63	7.80	39.83	17.00	11.76	5.38	1.29	6.05	6.05	32.35

[a] TRD

Appendix 7.3.4.2: Airomir+AeroChamber Plus Part 2 Study (with charcoal blockade)

Salbutamol urinary excretion of individual healthy volunteers						Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	13.2	61.2	37.0	48.1	11.0	13.7	18.7	72.4	181.3	109.0	54.5	47.7
2	8.5	60.6	31.1	52.1	21.1	13.1	19.4	77.0	180.6	103.5	51.8	42.9
3	11.9	52.7	33.6	40.8	7.2	20.1	17.3	80.0	182.7	102.7	51.4	50.0
4	14.8	71.2	45.1	56.4	11.3	17.0	21.5	77.0	178.5	101.4	50.7	30.2
5	13.7	63.4	43.6	49.7	6.2	15.4	18.9	80.8	181.1	100.3	50.1	26.8
6	9.8	45.3	27.5	35.5	8.1	19.6	17.7	73.6	182.3	108.7	54.4	63.4
7	21.3	72.6	43.5	51.4	7.9	17.2	22.7	77.9	177.3	99.4	49.7	16.8
8	15.5	71.4	47.9	55.9	8.0	15.0	20.4	83.7	179.6	96.0	48.0	24.6
9	16.1	71.6	46.7	55.5	8.8	12.8	16.8	77.5	183.2	105.7	52.9	34.1
10	13.6	53.6	33.4	40.0	6.6	17.6	20.3	82.0	179.7	97.7	48.9	44.2
11	9.6	41.7	27.5	32.1	4.6	17.8	24.0	78.9	176.0	97.1	48.5	55.4
12	16.6	67.3	45.9	50.8	4.8	15.2	20.7	81.1	179.3	98.1	49.1	30.8
13	21.2	76.7	47.5	55.6	8.1	14.5	19.7	84.0	180.3	96.3	48.2	11.6
Mean	14.28	62.28	39.25	47.99	8.74	16.08	19.86	78.91	180.14	101.23	50.62	36.80
SD	3.98	11.09	7.80	8.21	4.20	2.35	2.10	3.52	2.10	4.46	2.23	15.30
RSD	27.85	17.80	19.88	17.11	47.98	14.64	10.55	4.46	1.16	4.40	4.40	41.57

[a] TRD

Appendix 8.2.6.1: APSD of two puffs (200 µg) of Salamol + Volumatic obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	8.76	7.51	8.24	7.00	5.84	7.47	1.13	15.16	7.51
MDI Actuator	22.85	21.51	18.87	18.16	18.29	19.94	2.12	10.63	18.87
Spacer	75.62	70.46	84.93	81.79	75.64	77.69	5.70	7.34	75.64
ACI Throat	7.99	4.79	5.75	5.10	5.73	5.87	1.25	21.33	5.73
ACI S-0	2.40	2.55	2.56	1.96	2.31	2.36	0.24	10.39	2.40
ACI S-1	4.76	4.46	4.25	3.88	5.26	4.52	0.52	11.55	4.46
ACI S-2	6.08	7.98	7.25	6.01	6.37	6.74	0.85	12.62	6.37
ACI S-3	21.30	23.79	23.53	23.26	17.61	21.90	2.59	11.83	23.26
ACI S-4	33.88	34.09	28.81	31.98	34.71	32.69	2.40	7.33	33.88
ACI S-5	22.05	19.14	18.00	18.55	20.20	19.59	1.60	8.17	19.14
ACI S-6	4.43	7.32	3.45	4.09	5.00	4.86	1.49	30.61	4.43
ACI S-7	2.16	2.77	1.63	1.93	2.17	2.13	0.42	19.63	2.16
ACI Filter	2.35	2.34	2.35	2.54	2.37	2.39	0.08	3.55	2.35
Total Recovery (µg)	214.64	208.70	209.62	206.24	201.50	208.14	4.81	2.31	208.70
% Recovery[a]	107.32	104.35	104.81	103.12	100.75	104.07	2.40	2.31	104.35
Mass Balance[b] (µg)	205.88	201.19	201.38	199.24	195.66	200.67	3.71	1.85	201.19
% Recovery	102.94	100.60	100.69	99.62	97.83	100.33	1.85	1.85	100.60

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 8.2.6.2: APSD of two puffs (200 µg) of Salamol + Aerochamber Plus obtained with ACI at 28.3 ± 1.5 L/min

Identity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	SD	RSD	Median
MDI Canister Valve	11.33	13.57	11.92	14.69	11.67	12.64	1.44	11.36	11.92
MDI Actuator	19.62	20.96	21.88	24.29	19.22	21.19	2.03	9.58	20.96
Spacer	88.98	84.69	92.80	76.10	79.10	84.34	6.87	8.14	84.69
ACI Throat	3.58	4.63	4.79	4.47	4.42	4.38	0.47	10.75	4.47
ACI S-0	0.84	1.44	0.93	1.13	1.17	1.10	0.23	21.12	1.13
ACI S-1	1.49	1.49	1.39	1.02	1.68	1.41	0.24	17.32	1.49
ACI S-2	2.24	2.21	2.59	3.42	2.91	2.67	0.51	18.93	2.59
ACI S-3	19.16	14.79	17.21	26.10	25.57	20.56	5.06	24.59	19.16
ACI S-4	30.48	34.71	32.00	36.64	37.15	34.19	2.90	8.47	34.71
ACI S-5	28.52	24.46	20.60	18.61	15.46	21.53	5.09	23.65	20.60
ACI S-6	3.86	4.01	4.87	5.00	7.64	5.08	1.52	29.93	4.87
ACI S-7	1.68	2.17	1.77	1.83	2.33	1.96	0.28	14.37	1.83
ACI Filter	2.22	1.86	2.31	2.57	2.70	2.33	0.33	13.95	2.31
Total Recovery (µg)	214.00	210.99	215.06	215.86	211.01	213.38	2.27	1.07	214.00
% Recovery[a]	107.00	105.49	107.53	107.93	105.51	106.69	1.14	1.07	107.00
Mass Balance[b] (µg)	202.67	197.42	203.14	201.17	199.34	200.75	2.38	1.18	201.17
% Recovery	101.33	98.71	101.57	100.58	99.67	100.37	1.19	1.18	100.58

a = % Recovery calculated with respect to Nominal Dose (ND), i.e., two actuations of 100 µg per puff
b = Mass Balance = Ex-Canister Valve recovery. Recovery calculated with respect to Nominal Dose (ND) excluding deposition on Canister Valve but including deposition on Actuator (mouth piece).

Appendix 8.3.4.1: Salamol+Volumatic Part 1 Study (without charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	15.5	72.7	52.3	57.1	4.9	8.2	17.4	72.5	182.6	110.1	55.1	37.4
2	17.3	94.7	72.0	77.3	5.3	9.3	19.6	64.2	180.4	116.2	58.1	21.5
3	26.5	75.4	42.9	48.9	6.0	8.9	18.7	75.3	181.3	106.0	53.0	30.6
4	16.7	81.0	62.0	64.3	2.3	10.8	22.7	80.1	177.3	97.2	48.6	16.2
5	24.1	98.0	58.1	73.9	15.9	6.7	14.8	63.9	185.2	121.3	60.6	23.2
6	26.5	88.3	57.4	61.8	4.4	9.8	21.8	68.5	178.2	109.7	54.8	21.4
7	16.0	86.4	66.0	70.4	4.4	10.1	21.3	78.1	178.7	100.6	50.3	14.2
8	12.1	92.2	67.4	80.1	12.6	9.3	19.5	66.0	180.5	114.5	57.2	22.3
9	27.5	83.3	52.2	55.9	3.6	11.2	23.6	77.3	176.4	99.2	49.6	15.8
10	12.8	69.9	42.3	57.2	14.9	11.4	24.1	76.2	175.9	99.7	49.8	29.8
11	14.9	79.0	46.6	64.2	17.5	6.7	15.9	80.2	184.1	103.9	51.9	24.8
12	14.4	87.7	61.5	73.3	11.8	9.9	20.7	69.2	179.3	110.0	55.0	22.3
13	28.8	104.0	67.1	75.2	8.1	8.2	18.2	67.6	181.8	114.2	57.1	10.2
Mean	19.48	85.59	57.52	66.12	8.60	9.26	19.87	72.25	180.13	107.88	53.94	22.29
SD	6.16	10.07	9.69	9.67	5.24	1.52	2.85	5.97	2.85	7.49	3.74	7.39
RSD	31.64	11.76	16.85	14.62	60.92	16.42	14.33	8.26	1.58	6.94	6.94	33.15

[a] TRD

Appendix 8.3.4.2: Salamol+AeroChamber Plus Part 1 Study (without charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	17.9	93.1	67.1	75.3	8.2	9.1	15.5	78.7	184.5	105.8	52.9	12.6
2	10.4	69.1	45.4	58.7	13.2	12.8	21.9	94.6	178.1	83.5	41.7	14.4
3	27.2	105.1	68.8	77.9	9.2	8.7	14.8	72.3	185.2	112.9	56.4	7.7
4	12.5	80.4	52.7	67.9	15.2	12.4	21.3	88.1	178.7	90.6	45.3	10.2
5	11.4	74.1	39.7	62.7	22.9	10.2	18.3	92.8	181.7	88.9	44.5	14.8
6	18.4	84.9	62.9	66.6	3.6	9.8	16.2	85.8	183.8	98.0	49.0	13.1
7	25.7	103.5	66.2	77.8	11.6	9.3	15.4	71.1	184.6	113.4	56.7	10.0
8	26.5	108.0	72.7	81.5	8.8	9.4	15.5	67.0	184.5	117.5	58.7	9.5
9	11.7	71.1	42.5	59.4	16.9	13.1	25.4	86.8	174.6	87.9	43.9	16.8
10	20.6	95.7	69.6	75.1	5.5	11.0	21.4	68.7	178.6	109.9	54.9	14.1
11	18.5	84.4	56.6	65.9	9.3	10.9	19.8	86.2	180.2	94.0	47.0	9.6
12	13.9	77.4	52.6	63.6	11.0	9.5	17.2	90.2	182.8	92.5	46.3	15.1
13	14.1	77.6	49.4	63.5	14.1	9.5	16.2	89.2	183.8	94.6	47.3	17.1
Mean	17.59	86.50	57.40	68.91	11.50	10.44	18.38	82.43	181.62	99.19	49.60	12.70
SD	5.94	13.32	11.19	7.67	5.08	1.49	3.28	9.61	3.28	11.29	5.64	3.02
RSD	33.76	15.39	19.50	11.14	44.20	14.26	17.84	11.66	1.81	11.38	11.38	23.78

[a] TRD

Appendix 8.3.4.3: Salamol+Volumatic Part 2 Study (with charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	20.2	67.5	42.7	47.3	4.7	9.9	20.0	69.3	180.0	110.7	55.3	87.5
2	23.3	83.3	52.1	60.0	7.9	9.7	19.5	72.6	180.5	107.9	53.9	102.8
3	15.1	60.4	37.7	45.4	7.6	8.5	17.9	81.1	182.1	101.0	50.5	78.4
4	21.0	78.8	51.3	57.8	6.5	9.8	19.4	77.2	180.6	103.4	51.7	98.1
5	12.9	56.4	33.9	43.5	9.6	8.7	17.3	82.3	182.7	100.5	50.2	73.7
6	15.7	63.5	40.6	47.9	7.2	10.2	20.2	71.9	179.8	107.9	53.9	83.7
7	16.2	62.5	37.7	46.3	8.7	10.3	20.5	75.2	179.5	104.4	52.2	83.0
8	18.5	76.6	44.4	58.1	13.7	9.2	17.9	74.1	182.1	107.9	54.0	94.5
9	16.9	65.3	36.6	48.3	11.7	9.6	18.6	83.2	181.4	98.2	49.1	83.9
10	16.3	62.9	38.7	46.5	7.9	10.4	20.2	72.9	179.8	106.9	53.4	83.1
11	14.5	49.0	26.2	34.4	8.2	9.3	19.1	86.2	180.9	94.7	47.3	68.0
12	20.7	73.0	43.2	52.4	9.2	11.3	20.7	64.2	179.3	115.1	57.5	93.8
13	16.6	60.4	34.7	43.8	9.1	11.6	21.3	69.5	178.7	109.3	54.6	81.7
Mean	17.54	66.12	39.98	48.59	8.61	9.89	19.43	75.35	180.57	105.22	52.61	85.56
SD	3.00	9.58	6.99	7.01	2.25	0.91	1.21	6.35	1.21	5.57	2.78	9.75
RSD	17.12	14.48	17.49	14.43	26.20	9.24	6.24	8.43	0.67	5.29	5.29	11.39

[a] TRD

Appendix 8.3.4.4: Salamol+AeroChamber Plus Part 2 Study (with charcoal blockade)

	Salbutamol urinary excretion of individual healthy volunteers					Dose retained in MDI device components and spacer						TDD-TRD
Subj	**USAL0.5**	**USAL24[a]**	**USAL24Pre**	**USAL24Post**	**USALMET**	**Canister**	**Actuator**	**SP**	**TED**	**TDD**	**TDD %ND**	
1	14.5	61.8	37.9	47.4	9.5	12.7	21.2	81.6	178.8	97.2	48.6	35.4
2	18.4	63.6	41.0	45.1	4.2	10.7	17.8	80.2	182.2	101.9	51.0	38.4
3	24.1	77.8	47.2	53.7	6.5	13.3	22.2	79.0	177.8	98.7	49.4	20.9
4	15.8	64.6	32.9	48.8	15.9	10.5	17.6	85.2	182.4	97.2	48.6	32.6
5	14.0	54.4	30.1	40.5	10.3	13.7	23.4	87.0	176.6	89.6	44.8	35.1
6	10.1	49.5	28.3	39.3	11.0	14.4	24.6	88.3	175.4	87.2	43.6	37.7
7	16.4	64.7	40.8	48.4	7.5	12.9	22.1	82.5	178.0	95.4	47.7	30.7
8	25.8	81.8	47.7	56.0	8.3	9.8	16.2	83.1	183.8	100.7	50.4	18.9
9	19.5	67.4	42.0	48.0	6.0	10.4	17.2	90.6	182.8	92.3	46.1	24.8
10	13.8	55.5	28.2	41.8	13.5	11.7	19.2	94.1	180.8	86.7	43.3	31.1
11	12.3	53.0	30.1	40.7	10.6	11.3	17.3	95.5	182.7	87.3	43.6	34.3
12	11.9	47.1	31.0	35.2	4.2	13.3	24.3	86.8	175.8	89.0	44.5	41.9
13	18.7	65.8	41.9	47.1	5.2	13.9	23.6	81.8	176.4	94.6	47.3	28.8
Mean	16.55	62.08	36.85	45.52	8.67	12.20	20.52	85.81	179.48	93.67	46.84	31.60
SD	4.65	10.25	7.06	5.87	3.59	1.53	3.05	5.19	3.05	5.36	2.68	6.79
RSD	28.11	16.51	19.16	12.90	41.34	12.56	14.86	6.04	1.70	5.72	5.72	21.49

[a] TRD